


525 Rec'd PCT/PTO 05 JAN 2001

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| FORM PTO-1350 (Rev. 12-29-99) | | U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE | ATTORNEY'S DOCKET NUMBER 003300-723 |
| TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371 | | | U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5) 09/743107 |
| INTERNATIONAL APPLICATION NO. PCT/SE99/01230 | INTERNATIONAL FILING DATE 6 July 1999 | PRIORITY DATE CLAIMED 6 July 1998, 17 July 1998, 29 December 1998 | |
| TITLE OF INVENTION PEPTIDES BASED ON THE SEQUENCE OF HUMAN LACTOFERRIN AND THEIR USE | | | |
| APPLICANT(S) FOR DO/EO/US LARS Å. HANSON, LARS BALTZER, INGER MATTSBY-BALTZER and GUNNAR T. DOLPHIN | | | |
| <p>Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information: It is contemplated that this Application be prosecuted in the United States while using Claims 1 to 52 that were filed on September 29, 2000 during the international phase of the examination as further amended in the Preliminary Amendment filed herewith.</p> <p>1. <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.</p> <p>2. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.</p> <p>3. <input checked="" type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and the PCT Articles 22 and 39(1).</p> <p>4. <input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.</p> <p>5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2))</p> <p>a. <input checked="" type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau).</p> <p>b. <input checked="" type="checkbox"/> has been transmitted by the International Bureau.</p> <p>c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US)</p> <p>6. <input type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)).</p> <p>7. <input type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))</p> <p>a. <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau).</p> <p>b. <input type="checkbox"/> have been transmitted by the International Bureau.</p> <p>c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired.</p> <p>d. <input type="checkbox"/> have not been made and will not be made.</p> <p>8. <input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).</p> <p>9. <input checked="" type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)) (signed Declaration will follow).</p> <p>10. <input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).</p> <p>Items 11. to 16. below concern other document(s) or information included:</p> <p>11. <input checked="" type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98.</p> <p>12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.</p> <p>13. <input checked="" type="checkbox"/> A FIRST preliminary amendment.</p> <p><input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment.</p> <p>14. <input type="checkbox"/> A substitute specification.</p> <p>15. <input type="checkbox"/> A change of power of attorney and/or address letter.</p> <p>16. <input checked="" type="checkbox"/> Other items or information: Small Entity status is claimed.</p> <p>Certified copies of Swedish Application No. 9802441-7 filed 6 July 1998, Swedish Application No. 9802562-0 filed 17 July 1998, and Swedish Application No. 9804614-7 filed 29 December 1998 were submitted to the international office. Thus it is believed that the claims for priority have been perfected.</p> | | | |

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|---|--------------|--|------------------|---|--------------|
| U.S. APPLICATION NO. (If known, use 37 CFR 1.501) 09/743107 | | INTERNATIONAL APPLICATION NO. PCT/SE99/01230 | | ATTORNEY'S DOCKET NUMBER 003300-723 | |
| 17. <input type="checkbox"/> The following fees are submitted: | | | | CALCULATIONS | PTO USE ONLY |
| Basic National Fee (37 CFR 1.492(a)(1)-(5)): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO \$1,000.00 (960) International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$860.00 (970) International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$710.00 (958) International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$690.00 (956) International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) \$100.00 (962) <div style="text-align: right;">ENTER APPROPRIATE BASIC FEE AMOUNT =</div> | | | | \$ 1,000.00 | |
| Surcharge of \$130.00 (154) for furnishing the oath or declaration later than 20 <input type="checkbox"/> 30 <input type="checkbox"/> months from the earliest claimed priority date (37 CFR 1.492(e)). <div style="text-align: right;">\$</div> | | | | | |
| Claims | Number Filed | Number Extra | Rate | | |
| Total Claims | 42 -20 = | 22 | X\$18.00 (966) | \$ 396.00 | |
| Independent Claims | 11 -3 = | 8 | X\$80.00 (964) | \$ 240.00 | |
| Multiple dependent claim(s) (if applicable) | | | + \$270.00 (968) | \$ | |
| TOTAL OF ABOVE CALCULATIONS = | | | | \$ 1,636.00 | |
| Reduction for 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28). | | | | \$ 818.00 | |
| SUBTOTAL = | | | | \$ 818.00 | |
| Processing fee of \$130.00 (156) for furnishing the English translation later than 20 <input type="checkbox"/> 30 <input type="checkbox"/> months from the earliest claimed priority date (37 CFR 1.492(f)). | | | | \$ | |
| TOTAL NATIONAL FEE = | | | | \$ 818.00 | |
| Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 (581) per property + | | | | \$ | |
| TOTAL FEES ENCLOSED = | | | | \$ 818.00 | |
| | | | | Amount to be: | |
| | | | | refunded | \$ |
| | | | | charged | \$ |
| a. <input checked="" type="checkbox"/> A check in the amount of \$ <u>818.00</u> to cover the above fees is enclosed. b. <input type="checkbox"/> Please charge my Deposit Account No. <u>02-4800</u> in the amount of \$ _____ to cover the above fees. A duplicate copy of this sheet is enclosed. c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <u>02-4800</u> . A duplicate copy of this sheet is enclosed. NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status. SEND ALL CORRESPONDENCE TO: <div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> Benton S. Duffett, Jr. BURNS, DOANE, SWECKER & MATHIS, L.L.P. P.O. Box 1404 Alexandria, Virginia 22313-1404 (703) 836-6620 </div> <div style="width: 45%; text-align: center;">  SIGNATURE Benton S. Duffett, Jr. NAME <u>22,030</u> REGISTRATION NUMBER </div> </div> <div style="margin-top: 10px;"> Filed: January 5, 2001 </div> | | | | | |

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of)
LARS Å. HANSON et al.)
Application No.: (unassigned)) Box: PCT
Filed: January 5, 2001) Attention: DO/EO/US
For: PEPTIDES BASED ON THE) Examiner: (unassigned)
SEQUENCE OF HUMAN)
LACTOFERRIN AND THEIR USE) Group Art Unit: (unassigned)

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

This is a national phase filing of International Application No. PCT/SE99/01230,
filed 6 July 1999.

It is contemplated that this Application be prosecuted in the United States while
using Claims 1 to 52 that were submitted on September 29, 2000 during the international
phase of examination as further amended as indicated hereafter.

In the Abstract:

Please add the Abstract of the Disclosure that is provided on a separate sheet.

In the Claims:

Claim 12, line 1, delete "or 11".

Claim 22, line 2, delete "any of the claims 1-21" and insert --claim 1--.

Claim 27, lines 1 and 2, delete "any one of the claims 22-27" and insert

--claim 22--.

Claim 28, lines 1 and 2, delete "any one of the claims 22-27" and insert

--claim 22--.

Claim 30, lines 1 and 2, delete "any one of the claims 22-29" and insert

--claim 22--.

Claim 31, lines 1 and 2, delete "any one of the claims 1-21" and insert --claim 1--.

Please cancel "use" Claims 33 to 42 without prejudice.

Please add the following new Claim 53:

--53. A peptide according to claim 11, wherein the N-terminal amino acid is acetylated and/or the C-terminal amino acid is amidated.--

REMARKS

The present Amendment provides an Abstract of the Disclosure on a separate sheet, eliminates the use of multiple dependency, and cancels Claims 33 to 42 without prejudice.

The examination and allowance of the Application are respectfully requested.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

By: Benton S. Duffett, Jr.
Benton S. Duffett, Jr.
Registration No. 22,030

P.O. Box 1404
Alexandria, Virginia 22313-1404
(703) 836-6620

Filed: January 5, 2001

Abstract of the Disclosure

The invention relates to new peptides formed of at least seven subsequent amino acids of the amino acids in position 12-40, counted from the N-terminal end, in the sequence constituting human lactoferrin, and preferably modifications thereof. The invention also relates to medicinal products comprising such peptides, especially intended for treatment and prevention of infections, inflammations and tumours. Furthermore, the invention relates to food stuff, e.g. infant formula food, comprising the above mentioned peptides.

PEPTIDES BASED ON THE SEQUENCE OF HUMAN LACTOFERRIN AND THEIR USE

Field of the invention

The present invention relates to new peptides and to use thereof, in particular for treatment and/or prevention of infections, inflammations and/or tumours.

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Background art

It has for a long time been known that human milk in several ways is anti-inflammatory due to the fact that it is poor in initiators and mediators of inflammation but
10 rich in anti-inflammatory agents (see e.g. Goldman A. S., et al., Anti-inflammatory properties of human milk, Acta Paediatr. Scand. 75:689-695, 1986). Human milk also contains several soluble anti-infective components, such as
15 lactoferrin (see e.g. Hanson L. Å., et al., Protective factors in milk and the development of the immune system, Pediatrics 75:172-176, 1983).

Lactoferrin is a single chain metalbinding glycoprotein with a molecular weight of 77 kd. It has been found that the structural domain of lactoferrin responsible for
20 the bactericidal properties is a pepsin-cleaved fragment called lactoferricin (see e.g. Bellamy W., et al., Identification of the bactericidal domain of lactoferrin, Biochim. Biophys. Acta 1121:130-136, 1992, and Bellamy W., et al., Antibacterial spectrum of lactoferricin B, a
25 potent bactericidal peptide derived from the N-terminal region of bovine lactoferrin, J. Appl. Bact. 73:472-479, 1992).

Lactoferrin receptors are found on many types of cells including monocytes and macrophages, lectin-
30 stimulated human peripheral blood lymphocytes, brush-border cells, and tumour cell lines.

Several patent publications describe the possible use of lactoferrin for treatment of infections or inflammations. In WO 98/06425, e.g., it is disclosed that lac-

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toferrin and lactoferricin can be used for treatment and prevention of infections, inflammations and tumours.

EP-A-0 629 347 describes an antimicrobial agent containing (A) lactoferrin hydrolysate and/or one or more of antimicrobial peptides derived from lactoferrins, and (B) one or more compounds selected from the group consisting of metal-chelating protein, tocopherol, cyclodextrin, glycerine-fatty acid ester, alcohol, EDTA or a salt thereof, ascorbic acid or a salt thereof, citric acid or a salt thereof, polyphosphoric acid or a salt thereof, chitosan, cysteine, and cholic acid as the effective components thereof. This antimicrobial agent is intended for treatment of products, and especially for safely treating e.g. food and medicines. The agent according to this publication is thus a new preservative. In the publication several peptide sequences are given and some of them resemble the peptides according to the invention, although there are several important differences described further below.

Even though native human lactoferrin and lactoferricin have been shown to have desired anti-inflammatory, anti-infectious and anti-tumoural properties they cannot be used clinically on a broad basis since they are very expensive substances due to high production costs.

Summary of the invention

The object of the present invention is to provide new peptides which can be used for the same purposes as lactoferrin and/or lactoferricin and which will have the same, or better, effects although being much cheaper to produce.

The aim of the studies leading to the present invention was to design new peptides which could be taken up from the intestines. It has been shown that humans in their brush border membrane have receptors which can bind to human lactoferrin (see e.g. Lönnerdal B., Lactoferrin receptors in intestinal brush border membranes, Adv. Exp.

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the sequence constituting human lactoferrin, as well as modifications thereof.

According to a forth embodiment of the invention it is shown that modified peptides consisting of 12 aminoac-
5 ids based on the sequence consisting of the amino acids in positions 20-31 in human lactoferrin, counted from the N-terminal end, give even better results for the purposes of the present invention.

A plausible mechanism for the uptake of these new
10 peptides in the human body is that the peptides are taken up in the intestine through binding to the above mentioned specific lactoferrin receptors and are then transported through the circulation. However, the invention is in no way limited to this mechanism.

15 Thus, the present invention relates to new peptides with the sequences given in the appended sequence listing, and to functionally equivalent homologues or analogues thereof.

Furthermore, the invention relates to medicinal
20 products and to food stuff, especially infant formula food, comprising said peptides.

The invention also relates to use of said peptides for the production of medicinal products for treatment and prevention of infections, inflammations and tumours.

25 The peptides according to the invention are fungicidal and bactericidal, and can thus be used for other applications when substances with such properties are desired. They may for example be used as preservatives.

The characterising features of the invention will be
30 evident from the following description and the appended claims.

Detailed description of the invention

Thus, the present invention relates to peptides comprising - amino acid based on a fragment of the protein
35 human lactoferrin (hLF). The fragment of hLF that are

used as a basis for the invention is constituted by the amino acids in positions 12-40, the sequence of which is:

V-S-Q-P-E-A-T-K-C-F-Q-W-Q-R-N-M-R-K-V-R-G-P-P-V-S-C-I-K-R

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(SEQ. ID. NO. 100)

In the description single-letter symbols are used to denote the amino acids, while three-letter symbols are used in the appended sequence listing. These symbols, which are well known to man skilled in the art, have the following meaning: A = Ala = alanine, C = Cys = cysteine, D = Asp = aspartic acid, E = Glu = glutamic acid, F = Phe = phenylalanine, G = Gly = glycine, I = Ile = isoleucine, K = Lys = lysine, M = Met = methionine, N = Asn = asparagine, P = Pro = proline, Q = Gln = glutamine, R = Arg = arginine, S = Ser = serine, T = Thr = threonine, V = Val = valine, W = Trp = tryptophan and X = Xaa = a variable amino acid. Ac and NH₂ in some of the sequences denote an acetyl (CH₃CO-) group and an amino group, respectively, that have been used to modify the amino and the carboxy terminals of the peptides.

The peptides according to the invention may have either of a linear or a cyclic form, which is further explained below.

All the sequences mentioned herein with SEQ. ID. NO. 1-99 are given in the appended sequence listing.

The first embodiment of the invention relates to peptides the sequence of which is:

Ac-X-X-T-K-X-F-X-W-Q-R-X-M-R-K-V-R-X-X-X-X-X-X-X-X-NH₂

(SEQ ID. NO. 1)

wherein X in position 1 is either E or no amino acid, X in position 2 is either A or no amino acid, X in position 5 is either C or A, X in position 7 is either Q or K, X in position 11 is either N or D, and X in positions 17-25 are either no amino acids at all or -G-P-P-V-S-C-I-K-R.

The sequences for the peptides according to the first embodiment of the inventions are SEQ. ID. NO.1-7 in the appended sequence listing.

In a preferred version of the first embodiment of the invention X in position 1 is E, X in position 2 is A, X in position 5 is C, X in position 7 is Q, X in position 11 is N, and X in positions 17-25 are -G-P-P-V-S-C-I-K-R, which gives a peptide the sequence of which is SEQ. ID. NO. 2. The linear form is obtained through protection of the cysteine side chains by acetamidomethyl groups $\text{CH}_3\text{CONHCH}_2-$.

Another preferred version of the first embodiment of the invention is a cyclic form of SEQ. ID. NO. 2, obtained by the creation of a disulphide bridge between the two cysteines in positions 5 and 22, resulting in the cyclic peptide the sequence of which is SEQ. ID. NO. 3. The creation of the disulphide bridge has to be performed in a controlled way in order to avoid formation of polymers.

Another preferred version of the first embodiment of the invention is a somewhat shorter peptide wherein X in position 1 in SEQ. ID. NO. 1 is none, X in position 2 is none, X in position 5 is C, X in position 7 is Q, X in position 11 is N, and X in positions 17-25 are -G-P-P-V-S-C-I-K-R, resulting in a peptide the sequence of which is SEQ. ID. NO. 4.

Yet another preferred version of the first embodiment of the invention is a cyclic form of SEQ. ID. NO. 4 obtained by the creation of a disulphide bridge in the same way as for SEQ. ID. NO. 3, resulting in SEQ. ID. NO. 5.

An even more preferred version of this first embodiment of the invention is shorter peptide in which X in position 1 is none, X in position 2 is none, X in position 5 is A, X in position 7 is K, X₅ D, and X in positions 17-25 are none, resulting in SEQ. ID. NO. 6.

This peptide may also be modified so that the residues K in position 5 in SEQ. ID. NO. 6 and D in position

9 are linked by the formation of a lactam between the side chains of the residues, thus forming a loop. The sequence of this peptide is SEQ. ID. NO. 7. The lactam formation in this peptide between amino acid chains that are
5 four residues apart in the sequence forces the peptide to adopt a three-dimensional structure that resembles that of the fragment 18-31 of naturally occurring human lactoferrin and is designed to bind better to the receptor. This peptide with SEQ. ID. NO. 7 is the most preferred
10 peptide according to the first embodiment of the invention.

One advantage of the peptides with SEQ. ID. NO. 6 and SEQ. ID. NO. 7 compared to the other peptides according to the first embodiment of the invention is that they
15 are easier to synthesise and also cheaper per gram since they are shorter.

In all those seven peptides the amino and carboxy terminal ends have been capped, i.e. the free NH_2 group at the amino terminal end have been reacted with acetylimidazole to form the amide $\text{CH}_3\text{CONH-}$ or AcNH- and the
20 free COOH at the carboxy terminal end has been transformed into CONH_2 .

As evident from the sequences above all seven peptides according to the first embodiment of the invention
25 comprise the residues K and R at the carboxy terminal ends. These residues are positively charged under physiological conditions and are capable of strong and specific interactions with receptors. They are therefore an important part of the peptides according to the invention.
30 Also the T residue at the amino terminal end of all of the peptides according to the invention is capable of playing an important part in receptor binding.

The second embodiment of the invention relates to the peptide the sequence of which is:

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V-S-Q-P-E-A-T-K-C-F-Q-W-Q-R-N-M-R-K-V-R

(SEQ. ID. NO. 8)

and fragments thereof consisting of at least 7 amino acids. The sequences for the peptides according to the second embodiment of the inventions are SEQ. ID. NO.8-42 in the appended sequence listing.

The peptides according to this second embodiment of the invention contains at least 7 amino acids. Shorter peptides do not have the desired effects.

A preferred group of peptides according to the second embodiment of the invention are the peptides with SEQ. ID. NO. 9-22 given in the appended sequence listing. The advantage of these peptides, consisting of only seven amino acids each, is that they are relatively short which means that they are cheaper and more easy to produce than the longer peptides according to the invention.

Another preferred group of peptides according to this second embodiment of the invention are the peptides with SEQ. ID. NO. 13 and SEQ. ID. NO. 23-31 in the appended sequence listing, corresponding to modified sequences obtained from the amino acid in position 16 to the amino acid in position 22-31 of human lactoferrin, counted from the N-terminal end.

Yet another preferred group of peptides according to this embodiment are the peptides with SEQ. ID. NO. 22 and SEQ. ID. NO. 31-42 in the appended sequence listing, corresponding to modified sequences obtained from the amino acid in position 13-25 to the amino acid in position 31 of human lactoferrin, counted from the N-terminal end.

The advantage of the peptides according to the second embodiment of the invention is that they form the part of the lactoferricin fragment of the human lactoferrin protein, or a modified version thereof, which the inventors have found to be active with regards to the invention.

The third embodiment of the invention relates to peptides consisting of 11-17 amino acids, comprising the sequence:

F-X-W-X-R-X-M-R-K-X-R

(SEQ. ID. NO. 43)

5 or functionally equivalent homologues or analogues thereof. The sequences for the peptides according to the third embodiment of the inventions are SEQ. ID. NO.43-67 and SEQ. ID. NO. 97 in the appended sequence listing.

10 In this sequence, the amino acids denoted by X or Xaa are preferably, independently of each other, glutamine (Q or Gln), lysine (K or Lys), aspartic acid (D or Asp), asparagine (N or Asn), or valine (V or Val).

15 A preferred group of peptides according to the second embodiment of the invention consists of 14 amino acids. Those peptides correspond essentially to the sequence formed by the amino acids in positions 18-31, counted from the N-terminal end, in the sequence constituting human lactoferrin, wherein some amino acids have been modified. The peptides in this group have the sequences SEQ. ID. NO. 6, 7, 50-61 and 98.

20 The peptide according to this embodiment that mostly corresponds to this part of human lactoferrin is the peptide with SEQ. ID. NO. 50 given in the appended sequence listing. The capped version of this sequence has SEQ. ID. NO. 51.

25 The amino acid in position 3 in this sequence, i.e. a cysteine (C or Cys) may be replaced by an alanine (A or Ala) or a lysine, the amino acid in position 5, a glutamine, may be replaced by a lysine, the amino acid in position 9, an asparagine, may be replaced by an aspartic acid or a lysine, and the amino acid in position 13, a valine, may be replaced by an aspartic acid.

30 When the peptide according to this embodiment comprises a cysteine, as the peptides with SEQ. ID. NO. 46-51 and 62-67, it may be advantageous to replace this cysteine by an acetamidomethyl-cysteine in order to avoid that the peptide forms a disulphide bridge with another

peptide comprising a cysteine. However, the amino acids glutamine and valine may then not be replaced as described above.

5 A major advantage of the peptides according to this embodiment is that they form the part, or a modified version of it, of the lactoferricin fragment of the human lactoferrin protein which the inventors have found to be active with regards to the invention.

10 An other advantage of the peptides according to this embodiment is that they are relatively short which means that they are cheaper and easier to produce than longer peptides, such as lactoferrin itself.

15 The fourth embodiment of the invention relates to peptides consisting of 12 aminoacids. These peptides are based on a modification of the sequence consisting of the amino acids in positions 20-31 in human lactoferrin, counted from the N-terminal end, corresponding to SEQ. ID. NO. 46. The sequences for the peptides according to the third embodiment of the inventions are SEQ. ID. NO. 20 68-99 in the appended sequence listing. In the general sequence, SEQ. ID. NO. 99, Xaa in position 3 is preferably Gln or Ala, Xaa in position 4 is preferably Trp or Leu, Xaa in position 5 is preferably Gln, Lys, Orn, Ala or Nle, Xaa in position 6 is preferably Arg, Lys or Ala, 25 Xaa in position 7 is preferably Asn, Orn, Ala, or Nle, Xaa in position 8 is preferably Met or Leu, and Xaa in position 9 is preferably Arg or Lys. In some cases it may be advantageous to let this sequence be proceeded by the sequence Thr-Lys or the longer sequence Glu-Ala-Thr-Lys.

30 Preferred variants of the fourth embodiment of the invention are SEQ. ID. NO. 70 and SEQ. ID. NO. 74 wherein the amino acid in position 3 and position 7, respectively, in SEQ. ID. NO. 46 has been replaced by an alanine, SEQ. ID. NO. 81 and SEQ. ID. NO. 83 wherein the 35 amino acid in position 6 and position 9, respectively, in SEQ. ID. NO. 46 has been replaced by a lysine, and SEQ. ID. NO. 87 and SEQ. ID. NO. 89 wherein the amino acid in

position 5 and position 7, respectively, in SEQ. ID. NO. 46 has been replaced by an ornithine. It is also possible and in some cases preferable, to use capped versions of these sequences according to the invention.

5 The peptides according to the invention may be either of natural origin, e.g. derived from human lactoferrin, or synthetically produced.

10 The peptides according to the invention are sometimes "capped", so that the amino and the carboxy terminals of the peptides are turned into amides, as described above. The advantage of the capped versions, i.e. the sequences in which the amino and carboxy terminal ends have been reacted with acetylimidazole to form the amide $\text{CH}_3\text{CONH-}$ or AcNH- and the free COOH at the carboxy terminal end has been transformed into CONH_2 , is that these
15 peptides are neutral and uncharged and thus has drastically changed electrostatic properties. Assuming that the receptors bind the corresponding sequences of human lactoferrin where there are no N- and C terminal charges,
20 the capped peptides should bind better as they in this respect resemble the native protein more than uncapped peptides. Under physiological conditions at a pH of approximately 7, free amino and carboxy terminals would be ionised and the peptide would thus carry a positive and a
25 negative charge.

 In some cases only the capped form of a sequence has been given in the appended sequence listing. However, it is also possible, according to the invention, to use the non-capped forms.

30 When the peptide according to this embodiment comprises a lysine separated from an aspartic acid or a glutamic acid by three amino acids the lysine and the aspartic acid or the glutamic acid, respectively, may form a lactam, as in SEQ. ID. NO. 54, wherein a lactam is formed
35 between a lysine in position 5 and an aspartic acid in position 9, or as in SEQ. ID. NO. 55 wherein a lactam is formed between a lysine in position 5 and a glutamic acid

in position 9. It is also possible to obtain a dilactam, as e.g. in SEQ. ID. NO. 60 and SEQ. ID. NO. 61, wherein a lactam is formed between a lysine in position 3 and an aspartic acid in position 7 and another lactam is formed
5 between a lysine in position 9 and an aspartic acid in position 13. The lactam formation forces the peptide to adopt a three-dimensional structure that resembles that of the corresponding fragment of human lactoferrin and this may accomplish better binding of the peptide to the
10 lactoferrin receptor.

Apart from the above specified peptides it is also possible to use functionally equivalent homologues or analogues thereof, including those that mimic the three-dimensional structure of the corresponding segment in human lactoferrin due to the introduction of structural
15 constraints such as lactam bridges or other chemical constraints.

The peptides according to the invention are suitable for treatment and/or prevention of infections, inflammations and/or tumours. The term "treatment" used herein refers to curing, reversing, attenuating, alleviating, minimising, suppressing or halting the deleterious effects of a disease state, disease progression or other abnormal condition, and the term "prevention" used herein
20 refers to minimising, reducing or suppressing the risk of developing a disease state or progression or other abnormal or deleterious conditions.

The infections treatable with the peptides or medicinal products according to the invention include infections caused by all kinds of pathogens, such as bacteria, viruses, fungi, etc.
30

It is also possible to treat different types of inflammations. Inflammation is a complex phenomenon marked i.a. by abnormal "redness" and swelling of tissues and organs, pain and heat in affected areas, capillary dilation, leucocyte infiltration, etc. Inflammation is primarily caused by exposure to bacterial and other noxious
35

agents and physical injury. Inflammation has many forms and is mediated by a variety of different cytokines and other chemical signals. These mediators of inflammation include tumour necrosis factor- α (TNF- α), interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8), and various colony-stimulating factors (CSFs).

As stated above, the peptides according to the invention are also suitable for treatment of tumours.

The peptides according to the invention may either be used as they are or be included in a medicinal product or a pharmaceutical preparation. The medicinal product or a pharmaceutical preparation according to the invention may also comprise substances used to facilitate the production of the pharmaceutical preparation or the administration of the preparations. Such substances are well known to people skilled in the art and may for example be pharmaceutically acceptable adjuvants, carriers and preservatives.

The peptides or medicinal products according to the invention can be administered to a patient either systemically or locally. The term "patient" used herein relates to any person at risk for or suffering from a disease state, disease progression or other abnormal or deleterious condition.

The systemic administration is suitable e.g. for treatment of urinary tract infection, colitis and tumours. The systemic administration can be undertaken by oral, nasal, intravenous, intraartery, intracavitary, intramuscular, subcutaneous, transdermal, suppositories (including rectal) or other routes known to those of skill in the art. Oral administration is preferred.

The local administration is suitable e.g. for treatment of skin infections, all infections and inflammations in mucosal membranes etc. The local administration can be undertaken by topical, oral, nasal, vaginal or oropharyngeal route. For treatment of local infections or inflammations in the skin or mucosal membranes the peptides or

medicinal products according to the invention may e.g. be included in a gel, a cream, an ointment, or a paste.

In the method according to the invention an effective amount of a peptide according to the invention is administered to a patient. The term "effective amount" used herein relates an amount sufficient to treat or prevent a disease state, disease progression or other abnormal or deleterious conditions.

The peptides or medicinal products and methods according to the invention are particularly well suited for treatment and/or prevention of urinary tract infection and colitis, but several other inflammatory and infectious diseases are also treatable according to the present invention, such as inflammatory bowel diseases, rheumatoid arthritis, conditions caused by the virus HIV-1, conditions caused by the virus CMV, and conditions caused by the fungi *Candida albicans*, *Candida krusei* and *Cryptococcus neoformans*. This listing is in no way limiting the scope of the invention.

The peptides, medicinal products and methods according to the invention are also well suited for preventive medical care by reducing the risk of developing urinary tract infection or other inflammatory or infectious diseases in patients with an increased risk of attracting such complications.

The peptides, medicinal products and methods according to the invention may either be used alone, in combination with each other or in combination with conventional therapy.

According to the present invention it is also possible to include the peptides, in an effective amount, in any kind of food or beverage intended to reduce infections and/or inflammations in patients running an increased risk of such conditions due to an underlying disease, a low birth weight or a medical treatment. For example, it is possible to include the peptides, in an effective amount, in an infant formula food intended to in-

hibit harmful effects of bacteria, such as weight loss caused by inflammation induced by bacteria, viruses or fungi in infants. When the peptides according to the invention is to be used in food stuffs, e.g. for nutritional purposes, it is especially preferred to use peptides of natural origin.

Since the peptides according to the invention have antimicrobial effects they can also be used as preservatives in different food stuffs and medicinal products such as gels, creams, ointments, pastes, solutions, emulsions etc.

The invention will now be further explained in the following examples. These examples are only intended to illustrate the invention and should in no way be considered to limit the scope of the invention.

Brief description of the drawings

In the examples below, reference is made to the appended drawings on which:

Fig. 1 shows the number of bacteria (CFU) present in kidney in mice with urinary tract infection treated with two peptides according to the invention, peptide 3 and peptide 4, with human lactoferrin, hLF, and with water, respectively.

Fig. 2 illustrates the concentrations needed of peptide 3 in linear and cyclic form, peptide 4 in linear and cyclic form, and peptide 7 compared to a reference peptide in linear and cyclic form for killing of 99% of *C. albicans* at different pH.

Fig. 3 shows the number of *C. albicans* (CFU) present in stomach in mice, which were orally treated with two peptides according to the invention, peptide 3 and peptide 4, with human lactoferrin (hLF) and with water (Control), respectively, after intragastrical administration of *C. albicans*.

Fig. 4 illustrates the result of peroral treatment of mice with experimental colitis with peptide 3 and pep-

5 tide 4, and with hLF compared to treatment with water (control). Fig. 4 A shows the occurrence of occult blood in faeces, Fig. 4 B shows the amount of IL-1 β in serum, Fig. 4 C shows the occurrence of rectal bleeding, and Fig. 4 D shows the colon length.

Fig. 5 A illustrates the number of CD8 positive cells in tissue sections of distal colon from the mice treated according to Fig. 4.

10 Fig. 5 B illustrates the expression of MHC class II in tissue sections of distal colon from the mice treated according to Fig. 4.

Examples

15 In the examples below peptide 2 denotes the peptide according to the invention with SEQ. ID. NO. 2, peptide 3 denotes the peptide according to the invention with SEQ. ID. NO. 3, etc.

20 Morinaga 10, Morinaga 11, Morinaga 12, Morinaga 13, Morinaga 24 and Morinaga 25 denotes the peptides described in EP-A-0 629 347; Morinaga 10 is F-Q-W-Q-R-N (sequence No. 10 in EP-A-0 629 347), Morinaga 11 is F-Q-W-Q-R (sequence No. 11 in EP-A-0 629 347), Morinaga 12 is Q-W-Q-R (sequence No. 12 in EP-A-0 629 347), Morinaga 13 is W-Q-R (sequence No. 13 in EP-A-0 629 347), Morinaga 24 is the cyclic peptide with sequence K-C-F-Q-W-Q-R-N-M-R-K-V-R-G-P-P-V-S-C-I (sequence No. 24 in EP-A-0 629 347), and Morinaga 25 is K-C-F-Q-W-Q-R-N-M-R-K-V-R-G-P-P-V-S-C-I (sequence No. 25 in EP-A-0 629 347).

hLF denotes human lactoferrin.

30 In the examples, the minimal microbicidal concentrations (MMC) and minimal inhibitory concentrations (MIC) were determined as follows, unless otherwise specified in the examples. Bacterial or fungal strains were cultured in BHI medium over night at 37°C. A volume of the culture was transferred to a new tube with BHI and incubated for two more hours. Thereafter the cells were spun down and the pellet was suspended in BHI medium diluted 1/100 (1%

BHI). The concentration of bacterial or fungal cells was spectrophotometrically determined at 650 nm. The microbial concentrations were also determined by viable counts. Peptides serially diluted in 1% BHI by twofold or tenfold steps were added in triplicate to the wells of a microtiterplate (200 µl per well). The bacterial or fungal cell solutions were added in 10 µl volumes to give a final concentration of approximately $1-5 \times 10^5$ cells per ml in the well. The microplate was incubated at 37°C in a humid chamber for two hours. Thereafter 5 µl was taken from each well and added as a drop onto a blood agar plate and incubated over night at 37°C. The microplate was incubated for another 20 hours at 37°C and thereafter analysed spectrophotometrically at 650 nm in a microplate reader (Emax, Molecular Devices, USA). The concentration of peptide causing a 99% reduction of the inoculum after 2 hours of incubation was defined as the MMC_{99%}. The MIC value was defined as the concentration giving no increase in the absorbance value above the background level after 20 hours of incubation.

Example 1

This example illustrates solid phase synthesis of peptide 2, peptide 3, peptide 4 and peptide 5 according to the invention, and also of the peptides Morinaga 24 and Morinaga 25 used in the examples further below.

The syntheses were performed by Fmoc continuous flow strategy on a Biosearch Pioneer automated peptide synthesiser. The peptides were synthesised on a 0.1-0.2 mmol scale with the resins PAC-PEG-PS, 0.21 mmol/g for the peptide acids and Fmoc-PAL-PEG-PS, 0.20 mmol/g for the peptide amides.

The side chains of the peptides according to the invention were protected by piperidine-stable tert-butyl (for serine and threonine), tert-butyl ester (for glutamic acid), tert-butyloxycarbonyl (for lysine and tryptophan), triphenylmethyl (for asparagine, cysteine, glu-

tamine and histidine), acetamidomethyl (for cysteine), and 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulphonyl (for arginine) groups.

For the peptides Morinaga 24 and Morinaga 25, attachment of isoleucine to the PAC-PEG-PS resin was performed using the isoleucine symmetrical anhydride. The resin (1 g, 0.21 mmol) was allowed to swell in 3 ml dimethyl formamide (DMF). Fmoc-I (10 eq, 2.1 mmol) was dissolved in 5 ml dichloromethane (DMC) and 5 drops of DMF. Diisopropyl carbodiimide (DIPCDI) (5 eq, 1.05 mmol) was added to the amino acid solution after which it was left to stand for 20 min at 0°C. The DCM was removed under reduced pressure and the remaining oil was dissolved in DMF and added to the resin. Dimethylaminopyridine (DMAP) (1 eq) was added to the resin and the slurry was allowed to stand at room temperature with occasional swirling for 1 hour. After washing with DCM the resin was ready for peptide synthesis.

Removal of the α -amino protecting group (Fmoc) was performed with 20% piperidine in DMF for 7 min. All couplings proceeded in DMF, using 4 times excess of activated amino acid over peptide and 4 eq of benzotriazolyl tetramethyluronium tetrafluoroborate (TBTU):diisopropyl ethylamine (DIPEA) (1:2, mol/mol). A six fold excess of hydroxybenzotriazole (HOBt) was added to the couplings of the cysteine residues (acetamidomethyl and triphenylmethyl).

Peptide 2, peptide 3, peptide 4, and peptide 5 were acylated at the amino terminus using a 0.3 M solution of acetic anhydride in DMF.

Final deprotection and cleavage of the peptide from the resin was performed for 2 hours at room temperature using a mixture of 9.25 ml trifluoro acetic acid (TFA), 250 μ l water, 250 μ l ethanedithiol and 250 μ l triisopropylsilan per g of peptide resin. The resin was removed by filtration. The peptide was precipitated by use of cold diethylether, centrifuged and resuspended in fresh dieth-

ylether two more times to extract the scavengers and TFA. The peptides were dissolved in water and lyophilised.

The cyclisation of the disulphide bonded peptides, peptide 3 and peptide 5, were performed on unpurified material. Approximately 200 mg of peptide was dissolved in 1 l of degassed water. Ammonium hydrogen carbonate was added until the pH was in the range of 6-7 and the mixture was left with stirring and air contact for about 1 day, and was finally lyophilised.

The peptides were purified by reversed-phase high-pressure liquid chromatography eluting with isocratic mixtures of isopropanol (12-16% IPA) and 0.1% TFA. Two different columns were used, Microsorb, C-8 41.4x250 mm, 8 µm (column A), and Hichrom, C-8 25x250 mm, 7 µm (column B). The peptides eluted as broad peaks with the retention times (R) given in table 1 below. The identification was done by ES-MS. MW in the table denotes the molecular weight.

20 Example 2

This example illustrates solid phase synthesis of peptide 6 and peptide 7 according to the invention.

The syntheses were performed as described in example 1 with the following modifications of the synthetic procedure. The side chains of the lactam forming amino acids K in position 5 and D in position 9 were protected by 1(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl (Dde) and 4-{N-[1(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl]-amino}benzyl ester (ODmab), respectively.

After the synthesis of the capped sequence was completed, the Dde and ODmab groups were removed by 2% hydrazine (v/v) in DMF for 10 min. The resin was washed with DMF, 1M DIPEA in DMF and finally by DMF.

In the case of peptide 7 lactam formation between the side chains was allowed to proceed for 8 hours after the addition of a four fold excess of azabenzotriazolyl tetramethyluronium hexafluorophosphate (HATU):DIEPEA, 1:2

in DMF. The resin was washed with the following solvents/solutions: DMF, 20% piperidine in DMF, methanol and DCM. The resin was dried under vacuum, and the peptide was cleaved from the resin as described above.

- 5 Peptide 7 eluted as a broad peak with the retention times (R) given in table 1 below.

Table 1

| Peptide | Column | % IPA | Flow (ml/min) | R (min) | MW (found/calc.) |
|-------------|--------|-------|------------------|------------|------------------|
| Peptide 2 | B | 14 | 15 | 11 | 3201/3204.7 |
| Peptide 3 | A | 13 | 80 | 16 | 3057.1/3060.7 |
| Peptide 4 | A | 14 | 80 | 12 | 3002.4/3004.0 |
| Peptide 5 | B | 14 | 15 | 10 | not determined |
| Peptide 7 | B | 12 | 18 | 12 | not determined |
| Morinaga 24 | B | 16 | 15 | 20 | 2575/2576.0 |
| Morinaga 25 | B | 16 | 15 | 18 | 3430/3432.0 |

10

Example 3

In this example the bactericidal activity of the peptides according to the invention was tested and compared to the bactericidal activity of human lactoferrin.

- 15 Human lactoferrin (hLF), peptide 3, and peptide 4, respectively, were incubated with two different strains of E. coli, E. coli O14 (experiment I) and O6K5 (experiment II), in diluted growth medium (1/100 BHI - brain heart infusion) for 2 hours. The peptides were also incubated with E. coli O14 in 0.05 mM KCl (pH 7) without any growth medium (experiment III). Different concentrations of the peptides were tested.

- 20 After the incubation, samples were taken for bacterial plating. Serial dilutions of five- and fourfold steps were used in experiments I and II, respectively, and of twofold steps in experiment III.

25

The concentrations of the different peptides required for killing 100% (experiments I and II) or 90% (experiment III) of the bacteria are given in table 2.

5

Table 2

| Exp. | Agent | Concentration of agent for 100%/90% killing ($\mu\text{g/ml}$) |
|------|-----------|--|
| I | hLF | 400 |
| I | peptide 3 | 4.4 |
| I | peptide 4 | 4.4 |
| II | hLF | 2000 |
| II | peptide 4 | ≤ 7.8 |
| III | hLF | > 4000 |
| III | peptide 3 | 12.5 |
| III | peptide 4 | 3.2 |

From table 2 it is evident that the peptides according to the invention were much more efficient as bactericidal agents than human lactoferrin.

Example 4

In this example the fungicidal activity of the peptides according to the invention was tested and compared to the fungicidal activity of human lactoferrin. Different concentrations of hLF, peptide 3 and peptide 4 were incubated with different strains of *Candida albicans* and *Candida krusei* during 1-2 hours at 37°C in 0.05 mM KCl at pH 7.0 at two different occasions - experiment I and II, respectively. After incubation samples were taken for plating on Saboroud plates. The concentrations of the different peptides required for killing 99% of the fungi are given in table 3.

Table 3

| Exp. | Candida strain | Concentration of agent for 99% killing ($\mu\text{g/ml}$) | | | ratio hLF/ | |
|------|------------------------|---|-----------|-----------|------------|---------|
| | | hLF | peptide 3 | peptide 4 | /pep. 3 | /pep. 4 |
| I | C. albicans ATCC 64549 | > 50 | 0.6 | 1.25 | > 83 | > 40 |
| I | C. albicans CCUG 90028 | > 50 | 0.6 | 1.25 | > 83 | > 40 |
| I | C. krusei CCUG 35849 | 12.5 | 0.6 | 1.25 | 21 | 10 |
| I | C. krusei CCUG 969 | 25 | 1.25 | 2.5 | 20 | 10 |
| II | C. albicans ATCC 64549 | > 200 | 1.5 | 1.5 | > 133 | > 133 |
| II | C. albicans CCUG 599 | > 200 | 1.5 | 1.5 | > 133 | > 133 |
| II | C. albicans CCUG 1759 | > 200 | 1.5 | 1.5 | > 133 | > 133 |

The results in table 3 show that the peptides according to the invention were much more efficient fungicidal agents than human lactoferrin.

Example 5

In this example an in vitro test was performed to study the anti-inflammatory activity of the peptides according to the invention. More precisely, the inhibitory effect of the peptides according to the invention on the LPS-induced IL-6 response in a monocytic cell line (THP-1) was studied and compared to the effect of human lactoferrin by use of the method described by I. Mattsby-Baltzer et al., *Pediatr. Res.* 40:257-262, 1996. The IL-6 response in the THP-1 cells was induced by addition of LPS. hLF, peptide 3 and peptide 4, respectively, were added 30 minutes after LPS. A significant inhibition was

obtained with peptide 4, as shown in table 3 below. The inhibitory activity of peptide 3 was similar to the inhibitory activity of human lactoferrin.

5

Table 4

| Agent | % inhibition of LPS response |
|-----------|------------------------------|
| hLF | 15 |
| peptide 3 | 17 |
| peptide 4 | 39 |

Example 6

10 Peptide 3 and peptide 4 according to the invention were also tested in an in vivo study to show their effect on urinary tract infection.

15 E. coli 06K5 was instilled into the urinary bladder of mice. 30 minutes after instillation the different agents specified in table 5 were administered orally in an amount of 500 µg per mouse and 24 hours after instillation the number of bacteria (CFU) present in bladder and in kidney was determined. The result is shown in table 5.

20 The control group consisted of 10 animals in experiment I and of 23 animals in experiment II. The animals in the control groups were given tap water instead of peptide or hLF.

Table 5

| Exp | Agent | Mouse strain | No. of animals | Statistical comparison* of treatment group with control group of the number of bacteria, CFU, present in kidney |
|-----|-----------|--------------|----------------|---|
| I | peptide 4 | CH/HeN | 11 | p = 0.0137 |
| II | peptide 3 | C3H/Tif | 23 | p = 0.0574 |
| II | peptide 4 | C3H/Tif | 23 | p = 0.0102 |
| II | hLF | C3H/Tif | 23 | p = 0.006 |

* Mann-Whitney

5 The results from experiment II are also illustrated in figure 1.

Thus, the peptides according to the invention are capable of reducing the number of the bacteria in kidney.

10 Example 7

In this example an in vitro test was performed to compare the bactericidal and fungicidal activity of the peptides according to the invention with peptides described in EP-A-0 629 347. The peptide according to the invention used was peptide 4, and the peptides according to EP-A-0 629 347 called Morinaga 10, Morinaga 11, Morinaga 12, and Morinaga 13.

The peptides were incubated with E. coli 014 and Candida albicans. Two concentrations of C. albicans yeast cells were tested, $5 \cdot 10^6$ and $5 \cdot 10^3$ per ml. Different concentrations of the peptides were tested.

After the incubation, samples were taken for bacterial plating. Serial dilutions of tenfold steps were used in the experiments marked with I in table 5 and of two-fold steps in the experiments marked with II.

The concentrations of the different agents required for killing of 100% of the bacteria are given in table 6.

Table 6

5

| Agent | Concentration of agent for 100% killing ($\mu\text{g/ml}$) | | | |
|-------------|---|------|----------------|----------------|
| | E. coli O14 | | C. albicans | |
| | I | II | I | |
| | | | $5 \cdot 10^6$ | $5 \cdot 10^3$ |
| peptide 4 | > 10, <100 | 12 | > 10, <100 | 10 |
| hLF | nd | nd | > 1000 | > 2000 |
| Morinaga 10 | > 500 | 1000 | > 2000 | > 2000 |
| Morinaga 11 | > 500 | nd | > 2000 | > 2000 |
| Morinaga 12 | > 500 | nd | > 2000 | > 2000 |
| Morinaga 13 | > 500 | nd | > 2000 | > 2000 |

nd = not determined

10 From table 6 it is evident that the peptide according to the invention is a much more efficient bactericidal agent than the short peptides described in EP-A-0 629 347 and than human lactoferrin.

Example 8

15 The fungicidal and inhibitory activity of the peptide 2, peptide 3, peptide 4, and peptide 7 according to the invention were compared to the peptides described in EP-A-0 629 347 most resembling the peptides according to the invention, i.e. Morinaga 24 and Morinaga 25.

20 Candida albicans ATCC 64549 ($1 \cdot 10^5/\text{ml}$) was incubated in the presence of the peptides during 2 hours at 37°C in diluted growth medium (BHI, twofold serial dilutions starting with $50 \mu\text{g/ml}$). The fungicidal activity was measured by culturing $5 \mu\text{l}$ from each incubation well on Saboroud agar plates. The concentrations of the different
25 agents required for killing of 100% of the bacteria is given in table 7.

The inhibition of growth was measured spectrophotometrically after 20 hours of incubation. The concentration of agent needed for inhibition of growth is given in table 7.

5

Table 7

| Agent | Concentration of agent for 100% killing ($\mu\text{g/ml}$) | Concentration of agent for inhibition of growth ($\mu\text{g/ml}$) |
|-------------|--|--|
| Peptide 2 | 6.2 | 6.2 |
| Peptide 3 | 6.2 | 6.2 |
| Peptide 4 | 6.2 | 6.2 |
| Peptide 7 | 3.1 | 1.5 |
| Morinaga 24 | 12.5 | 12.5 |
| Morinaga 25 | 6.2 | 6.2 |

10 This example show that peptide 2, peptide 3, and peptide 4 according to the invention is more efficient with regard to fungicidal and inhibitory activity than the linear peptide Morinaga 24 and that peptide 7 according to the invention is an even better fungicidal agent and inhibitor of growth of fungi.

15

Example 9

Also the bactericidal and inhibitory activity of the peptide 2, peptide 3, peptide 4, and peptide 7 according to the invention were compared to the activities of Morinaga 24 and Morinaga 25.

20 E. coli 014 was incubated as described above in the presence of the peptides during 2 hours. The bactericidal activity was measured in the same way as described above. The concentrations of the different agents required for killing of 100% of the bacteria is given in table 8.

25

The inhibition of growth was measured spectrophotometrically after incubation during 20 hours. The con-

centration of agent needed for inhibition of growth is given in table 8.

Table 8

5

| Agent | Concentration of agent for 100% killing ($\mu\text{g/ml}$) | Concentration of agent for inhibition of growth ($\mu\text{g/ml}$) |
|-------------|--|--|
| Peptide 2 | 25 | 6.2 |
| Peptide 3 | 12.5 | 3.1 |
| Peptide 4 | 12.5 | 3.1 |
| Peptide 7 | 12.5 | ≤ 1.5 |
| Morinaga 24 | 25 | 6.2 |
| Morinaga 25 | 12.5 | 3.1 |

10 This example show that peptide 2, peptide 3 and peptide 4 according to the invention have bactericidal and inhibitory effects that are approximately the same as those for Morinaga 24 and Morinaga 25, but that peptide 7 is much more efficient with regards to inhibition of growth of bacteria.

Example 10

15 In this example, the microbicidal and microbiostatic activity of the peptides according to the invention were tested and compared to the two reference peptides.

20 C. albicans (ATCC64549) and E. coli O6, respectively, were incubated with the different peptides. The experiments were performed with 1, 10, 25 and 100 $\mu\text{g/ml}$ of peptide. The results are shown in table 9.

Table 9

| Peptide | C. albicans | | E. coli | |
|------------|--------------------|-----|--------------------|------|
| | MMC _{99%} | MIC | MMC _{99%} | MIC |
| Peptide 44 | 10 | 10 | 10 | 10 |
| Peptide 46 | 10 | 10 | 10 | 10 |
| Peptide 48 | 10 | 10 | 10 | 10 |
| Peptide 50 | 10 | 10 | 10 | 10 |
| Peptide 51 | 10 | 10 | 10 | 10 |
| Peptide 53 | 10 | 10 | 10 | 10 |
| Peptide 57 | 25 | 10 | 10 | 10 |
| Peptide 61 | 10 | 10 | 10 | 10 |
| Peptide 63 | 10 | 10 | 10 | 10 |
| Peptide 64 | 25 | 10 | 10 | 10 |
| Peptide 67 | 10 | 10 | 10 | 10 |
| Peptide 40 | 25 | 25 | > 25 | > 25 |
| Peptide 33 | 100 | 10 | 25 | 10 |

From the table, it is evident that the peptides according to the invention have very good microbicidal and microbiostatic activity, even though the shortest peptide, peptide 40, and the longest peptide, peptide 33, do not give as good results as the other peptides.

10 Example 11

In this example the activities of the peptides according to the invention on the killing of *C. albicans* and on the inhibition of the growth of *C. albicans* were studied.

15 *C. albicans* yeast cells (ATCC64549) were incubated for 2 hours at pH 4.5 in BHI medium diluted to 1% of the original concentration containing 25 µg/ml of the peptide. Thereafter the fungal solutions were cultured on blood agar plates. OD₆₅₀ was measured after incubation
20 during an additional 18 hours.

The fungicidal effect of the peptides on *C. albicans* was determined as the ability of the peptide to kill 100%

and 99%, respectively, of the fungus, while the growth inhibitory effect was determined by measuring the OD₆₅₀. An inhibitory effect existed when no increase in OD₆₅₀ was recorded. The results are shown in table 10.

5

Table 10

| Peptide | C. albicans | | |
|------------|-------------|-----|-------------|
| | killing: | | inhibition: |
| | 100% | 99% | |
| Peptide 44 | - | - | - |
| Peptide 46 | + | + | + |
| Peptide 48 | + | + | + |
| Peptide 50 | + | + | + |
| Peptide 51 | + | + | + |
| Peptide 6 | - | + | - |
| Peptide 57 | + | + | + |
| Peptide 61 | - | + | + |
| Peptide 62 | - | + | + |
| Peptide 64 | - | + | + |
| Peptide 66 | - | + | + |
| Peptide 40 | - | - | - |
| Peptide 33 | - | - | - |

killing: + = 100/99 % of the bacteria are killed
 - = less than 100/99% of the bacteria are killed

10

inhibition: + = no increase in OD₆₅₀ is seen
 - = OD₆₅₀ continues to increase

From the table, it is evident that the peptides according to the invention, especially Peptides 46, 48, 50, 51, and 57, have better effect on the killing and growth inhibition of C. albicans than the reference peptides.

15

Example 12

20

In this example, the peptides according to the invention were used to study the effect on the killing of

different bacteria. The different bacteria used are shown in table 11.

The peptides were used at a concentration of 25 µg/ml.

5 The results are shown in table 11.

Table 11

| Peptide | Bacteria | | | | |
|------------|----------------|-----------------|--------------|------------------|------------------|
| | E. faecalis | S. epidermis | S. aureus | K. pneumoniae | P. aeruginosa |
| Peptide 44 | + | + | + | - | - |
| Peptide 46 | + | + | + | + | + |
| Peptide 48 | + | + | + | + | + |
| Peptide 50 | + | + | + | + | + |
| Peptide 51 | + | + | + | + | + |
| Peptide 6 | + | + | + | + | + |
| Peptide 57 | + | + | + | + | + |
| Peptide 61 | + | + | + | + | + |
| Peptide 62 | + | + | + | + | + |
| Peptide 64 | + | + | + | - | - |
| Peptide 66 | + | + | + | - | - |
| Peptide 40 | - | + | - | - | - |
| Peptide 33 | + | + | + | - | - |

+ = 99% of the bacteria are killed

10 - = less than 99% of the bacteria are killed

From the table, it is evident that the peptides according to the invention, especially Peptides 46-62, have very good effect on the killing of bacteria, even though the shortest peptide, peptide 40, and the longest peptide, peptide 33, do not give as good results as the other peptides.

Example 13

20 In this example the concentrations needed of three peptides according to the invention for killing of 99% of

C. albicans at different pH was compared to a reference peptide. The peptides according to the invention were peptide 2 (linear), peptide 3 (cyclic), peptide 4 (linear), peptide 5 (cyclic) and peptide 7 (lactam bridged), all capped. The reference peptides used were an uncapped cyclic form and an uncapped linear form of a Morinaga peptide, Morinaga 24 and 25. The results are illustrated in Fig. 2. From the figure it is evident that the capped, linear peptides 3, and 4 as well as the lactam bridge containing peptide 7 have better effects than the capped cyclic peptides according to the invention, and that all the capped linear peptides according to the invention in addition to peptide 7 have better effects than the linear and cyclic, uncapped reference peptides (Morinaga 24 and 25) at pH 4.5. The most effective peptide at all different pH was peptide 7.

Example 14

In this example prevention of C. albicans colonisation in stomach was studied by giving human lactoferrin (HFL), the peptide with SEQ. ID. NO. 3 and the peptide with SEQ. ID. NO. 4 perorally to mice. A dose of 10^8 of C. albicans was given intragstrically to mice. Three days later HLF or peptide was given twice a day (in a total of 1 mg per day) for three days, and on the fourth day the mice were killed. The number of C. albicans in the stomach was determined by culturing and counting colony-forming units (CFU per ml). The results are illustrated in figure 3. It is evident from the results that the peptides effectively reduce the growth of C. albicans in the stomach.

Example 15

In this example the anti-inflammatory effects of the peptides according to the invention on experimental colitis in mice were studied. Acute colitis was induced in C57B1/6J mice by giving dextranulphate (5%) via the

drinking water (ad lib). Peptide 3 and peptide 4, as well as human lactoferrin (hLF), were orally administered to mice at the start of dextran sulphate treatment. The animals were killed on day 2.

5 It was found that blood in faeces of mice treated with the peptides according to the invention occurred in fewer animals than compared to the water-treated control group two days after treatment, as shown in Fig. 4 A.

10 Another indication of the fact that the peptides according to the invention are anti-inflammatory, is the reduced concentrations of the inflammatory cytokine IL-1 β present in sera from the mice treated according to the invention, as illustrated in Fig. 4 B, as well as the reduced occurrence of rectal bleeding, as shown in Fig. 4
15 C.

Another measurement of inflammation in the colon is the colon length - a shortened colon is inductive of inflammation. It was found, as shown in Fig. 4 D, that the colon of the mice treated according to the invention were
20 longer than the colon of the mice in the control group.

Thereafter the number of CD8- and MHC class II-positive cells in tissue sections of distal colon from the mice was studied, and the results are shown in Fig. 5. From Fig. 5 A, it is clear that the number of CD8-
25 positive T-cells in tissue sections of distal colon from the mice treated with the peptides according to the invention is significantly lower than for the water-treated control group.

From Fig. 5 B it is clear that the occurrence of
30 cells expressing MHC class II (macrophages, dendritic cells and B cells) in tissue sections of distal colon from the mice treated with the peptides according to the invention is higher than for the untreated control group, and similar to that of healthy animals with no acute colitis. These results indicate that mice treated with the
35 peptides according to the invention diminish or delay their local cellular response.

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33

Thus, the peptides according to the invention are effective in reducing the inflammation itself as well as the clinical symptoms.

5 Example 16

In this example the fungicidal activity of one of the peptides according to the invention, the peptide with SEQ. ID. NO. 7, was compared to the conventional antifungal agents flucytosine and fluconazole, and also to human lactoferrin (hLF). The antifungal agents were tested on C. albicans (ATCC 64549) in a concentration of 2×10^5 cells/ml. The tests were performed in BHI medium diluted 1/100. The results are shown in table 12.

15 Table 12

| Antifungal agent | MMC _{99%} (µg/ml) | MIC (µg/ml) |
|---------------------------|----------------------------|-------------|
| Flucytosine ^{*)} | > 500 | 250 |
| Fluconazole | > 500 | > 500 |
| hLF | > 1000 | 1000 |
| Peptide 7 | 6.2 | 6.2 |

^{*)} This may be inhibited by nonsynthetic media, however a 1/100 dilution of the medium used herein diminishes this risk.

20 It is evident from the results in table 12 that the peptide according to the invention is clearly more effective than the other substances.

Example 17

25 Also in this example one of the peptides according to the invention - this time the peptide with SEQ. ID. NO. 4, was compared to a conventional fungicide, amphotericin. The antifungal activity against C. albicans was studied. Twofold serial dilutions with a starting concentration of 20 µg/ml were used. BHI diluted 1/100 was used as growth medium.

30

Table 13

| Fungicidal agent | MMC _{99%} (µg/ml) | MIC (µg/ml) |
|------------------|----------------------------|-------------|
| Peptide 4 | 5.0 | 5.0 |
| Amphotericin | 2.5 | 0.6 |

It is evident from table 13 that the activity of the
5 peptide according to the invention is comparable to the
conventional fungicide.

Example 18

10 In this example the bactericidal activity of two of
the peptides according to the invention was studied in a
test with a multiresistant *S. aureus*. A bacterial solu-
tion with a concentration of 5.0×10^5 *S. aureus* bacte-
rial per ml was used. The concentration of each peptide
needed to kill 99% of the bacteria (MMC_{99%}) was determined
15 after 2 hours and after 24 hours of incubation at 37°C .
The results are illustrated in table 14 below.

Table 14

| SEQ. ID. NO. | MMC _{99%} (µg/ml) | |
|--------------|----------------------------|----------|
| | 2 hours | 24 hours |
| 4 | 5.0 | ≤ 0.32 |
| 48 | 5.0 | ≤ 0.32 |

20

Example 19

In this example the bactericidal activity of three
of the peptides according to the invention was studied in
a test with a reference strain of *S. aureus*. A bacterial
25 solution with a concentration of 5.0×10^5 *S. aureus* bac-
terial per ml was used. The concentration of each peptide
needed to kill 99% of the bacteria (MMC_{99%}) was determined
after 2 hours of incubation at 37°C. The lowest concen-
tration tested was 6.25 µg/ml. The results are illus-
30 trated in table 15 below.

Table 15

| SEQ. ID. NO. | MMC _{99%} (µg/ml) 2 hours |
|--------------|---------------------------------------|
| 36 | ≤ 6.25 |
| 98 | ≤ 6.25 |
| 7 | ≤ 6.25 |

5 Example 20

In this example a so called alanine scan of the peptide with SEQ. ID. NO. 46, C-F-Q-W-Q-R-N-M-R-K-V-R, was performed. In this alanine scan each amino acid was in turn substituted with an alanine, resulting in the peptides illustrated in table 16 below.

Table 16

| Exp. No. | amino acid in position | | | | | | | | | | | | SEQ. ID. NO. |
|----------|------------------------|---|---|---|---|---|---|---|---|---|---|---|--------------|
| 1 | A | F | Q | W | Q | R | N | M | R | K | V | R | 68 |
| 2 | C | A | Q | W | Q | R | N | M | R | K | V | R | 69 |
| 3 | C | F | A | W | Q | R | N | M | R | K | V | R | 70 |
| 4 | C | F | Q | A | Q | R | N | M | R | K | V | R | 71 |
| 5 | C | F | Q | W | A | R | N | M | R | K | V | R | 72 |
| 6 | C | F | Q | W | Q | A | N | M | R | K | V | R | 73 |
| 7 | C | F | Q | W | Q | R | A | M | R | K | V | R | 74 |
| 8 | C | F | Q | W | Q | R | N | A | R | K | V | R | 75 |
| 9 | C | F | Q | W | Q | R | N | M | A | K | V | R | 76 |
| 10 | C | F | Q | W | Q | R | N | M | R | A | V | R | 77 |
| 11 | C | F | Q | W | Q | R | N | M | R | K | A | R | 78 |
| 12 | C | F | Q | W | Q | R | N | M | R | K | V | A | 79 |

15 Example 21

In each of the experiments in this example one of the amino acids in positions 4, 6, 8 and 9 in the peptide with SEQ. ID. NO. 46 was replaced by a similar amino

acid. In experiment 1 the tryptophan in position 4 was replaced by a leucine, in experiment 2 the arginine in position 6 was replaced by a lysine, in experiment 3 the methionine in position 8 was replaced by a leucine, and in experiment 4 the arginine in position 9 was replaced by a lysine. The resulting peptides are illustrated in table 17.

Table 17

| Exp. No. | amino acid in position | | | | | | | | | | | | SEQ. ID. NO. |
|----------|------------------------|---|---|---|---|---|---|---|---|---|---|---|--------------|
| 1 | C | F | Q | L | Q | R | N | M | R | K | V | R | 80 |
| 2 | C | F | Q | W | Q | K | N | M | R | K | V | R | 81 |
| 3 | C | F | Q | W | Q | R | N | L | R | K | V | R | 82 |
| 4 | C | F | Q | W | Q | R | N | M | K | K | V | R | 83 |

Example 22

In each of the experiments in this example one of the amino acids in positions 5, 6, and 7 in the peptide with SEQ. ID. NO. 46 was replaced by a negatively charged amino acid. In experiment 1 the glutamine in position 5 was replaced by a glutamic acid, in experiment 2 the arginine in position 6 was replaced by glutamic acid, and in experiment 3 the asparagine in position 7 was replaced by glutamic acid. The resulting peptides are illustrated in table 18.

Table 18

| Exp. No. | amino acid in position | | | | | | | | | | | | SEQ. ID. NO. |
|----------|------------------------|---|---|---|---|---|---|---|---|---|---|---|--------------|
| 1 | C | F | Q | W | E | R | N | M | R | K | V | R | 84 |
| 2 | C | F | Q | W | Q | E | N | M | R | K | V | R | 85 |
| 3 | C | F | Q | W | Q | R | E | M | R | K | V | R | 86 |

Example 23

In each of the experiments in this example a neutral amino acid in the peptide with SEQ. ID. NO. 46 was replaced with either a positively charged amino acid or a neutral one. In experiment 1 the glutamine in position 5 was replaced by an ornithine, in experiment 2 the glutamine in position 5 was replaced by a norleucine, in experiment 3 the asparagine in position 7 was replaced by an ornithine, and in experiment 4 the asparagine in position 7 was replaced by a norleucine. The resulting peptides are illustrated in table 19.

Table 19

| Exp. No. | amino acid in position | | | | | | | | | | | | SEQ. ID. NO. |
|-------------|------------------------|---|---|---|-----|---|-----|---|---|----|----|----|-----------------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | |
| 1 | C | F | Q | W | Orn | R | N | M | R | K | V | R | 87 |
| 2 | C | F | Q | W | Nle | R | N | M | R | K | V | R | 88 |
| 3 | C | F | Q | W | Q | R | Orn | M | R | K | V | R | 89 |
| 4 | C | F | Q | W | Q | R | Nle | M | R | K | V | R | 90 |

Example 24

In each of the experiments in this example one or several of the amino acids in the peptide with SEQ. ID. NO. 46 was replaced with other amino acids. In experiment 1 the glutamine in position 5 was replaced with a lysine, in experiment 2 the glutamine in position 5 was replaced with an lysine and the asparagine in position 7 was replaced by an alanine, in experiment 3 the glutamine in position 3 was replaced with an alanine and the glutamine in position 5 was replaced with an lysine, in experiment 4 the glutamine in position 3 and the asparagine in position 7 were replaced with alanines, in experiment 5 the tryptophan in position 4 was replaced with a leucine, and the arginines in position 6 and position 9 were replaced with lysines, and in experiment 6 the glutamine in position 3 and the asparagine in position 7 were replaced

with alanine, the tryptophan in position 4 was replaced with a leucine, and the glutamine in position 5 and the arginines in positions 6 and 9 were replaced with lysines. The resulting peptides are illustrated in table 20.

5

Table 20

| Exp. No. | amino acid in position | | | | | | | | | | | | SEQ. ID. NO. |
|-------------|------------------------|---|---|---|---|---|---|---|---|----|----|----|-----------------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | |
| 1 | C | F | Q | W | K | R | N | M | R | K | V | R | 91 |
| 2 | C | F | Q | W | K | R | A | M | R | K | V | R | 92 |
| 3 | C | F | A | W | K | R | N | M | R | K | V | R | 93 |
| 4 | C | F | A | W | Q | R | A | M | R | K | V | R | 94 |
| 5 | C | F | Q | L | Q | K | N | M | K | K | V | R | 95 |
| 6 | C | F | A | L | K | K | A | M | K | K | V | R | 96 |

Example 25

- 10 In this example the fungicidal, and bactericidal effects of the different peptides obtained in examples 18-21 were studied. *C. albicans*, *E. coli* and *S. aureus*, respectively, were incubated in BHI medium diluted 1/100, with a pH of approximately 6.7-6.9. The concentration of
- 15 each peptide needed to kill 99% of the microorganisms ($MMC_{99\%}$) was determined. The results are illustrated in table 21 below.

Table 21

| SEQ. ID. NO. | MMC _{99%} | | |
|--------------|--------------------|---------|-----------|
| | C. albicans | E. coli | S. aureus |
| 46 | 12 | 12 | 7 |
| 68 | 12 | >25 | 28 |
| 69 | 12 | 12 | 14 |
| 70 | 6 | 6 | 7 |
| 71 | 12 | 25 | 14 |
| 72 | 12 | 12 | 7 |
| 73 | 12 | 12 | 7 |
| 74 | 6 | 6 | 3.5 |
| 75 | 25 | 12 | 7 |
| 76 | 25 | 25 | 7 |
| 77 | 25 | 12 | 7 |
| 78 | 25 | 12 | 7 |
| 79 | 25 | 12 | 7 |
| 80 | 6 | 12 | 7 |
| 81 | 6 | 6 | 7 |
| 82 | 6 | 12 | 7 |
| 83 | 6 | 6 | 7 |
| 84 | 12 | 25 | 14 |
| 85 | >50 | >25 | >28 |
| 86 | 25 | 25 | 14 |
| 87 | 3 | 6 | ≤3.5 |
| 88 | 6 | 12 | 3.5 |
| 89 | 6 | 6 | 3.5 |
| 90 | 12 | 12 | 3.5 |

Example 26

5 In this example the fungicidal, and bacteri-
 cidal effects of the different peptides obtained in exam-
 ple 22 were studied. C. albicans, E. coli and S. aureus,
 respectively, were incubated in BHI medium diluted 1/100,
 with a pH of approximately 6.7-6.9. The concentration of
 10 each peptide needed to kill 99% of the microorganisms

(MMC_{99%}) was determined. The results are illustrated in table 22 below.

Table 22

5

| SEQ. ID. NO. | MMC _{99%} | | |
|------------------|--------------------|---------|-----------|
| | C. albicans | E. coli | S. aureus |
| 46 | 12 | 6 | 12 |
| 91 ^{*)} | 6 | 6 | 6 |
| 93 | 12 | 6 | 6 |
| 94 | 12 | 6 | 6 |
| 95 | 12 | 6 | 6 |
| 96 | 12 | 6 | 12 |
| 97 | 12 | 6 | 6 |

^{*)} The peptide with SEQ. ID. NO. 91 was tested twice, and the results were the same at both times.

Example 27

10

In this example the fungicidal activity of three of the peptides according to the invention was studied, and tested against three different fungi. The fungi were incubated in BHI medium diluted 1/100. The concentration of each peptide needed to kill 99% of the fungi (MMC_{99%}) was determined. The results are illustrated in table 23 below.

15

Table 23

| SEQ. ID. NO. | MMC _{99%} | | |
|--------------|--------------------|-------------|---------------|
| | C. albicans | C. glabrata | C. neoformans |
| 4 | 6.3 | >50 | ≤3.1 |
| 46 | 12.5 | 50 | ND |
| 87 | 6.3 | >50 | 3.1 |

20

Example 28

In this example the microbicidal activity of eight of the peptides according to the invention was studied, and tested against *E. coli* O6K5 and *C. albicans*.

- 5 In this example, a peptide with SEQ. ID. NO. 55 was used, which has not been described above. This peptide is a modification of the peptide with SEQ. ID. NO. 7 wherein the Asp in position 9 is substituted with a Glu.

10 The concentration of each peptide needed to kill 99% of the microorganisms (MMC_{99%}) was determined. The results are illustrated in table 24 below.

Table 24

| SEQ. ID. NO. | MMC _{99%} | |
|--------------|--------------------|--------------------|
| | <i>E. coli</i> | <i>C. albicans</i> |
| 4 | 20 | 5 |
| 7 | > 20 | 5 |
| 55 | 5 | 5 |
| 46 | 10 | 10 |
| 87 | 5 | 5 |
| 88 | 5 | 10 |
| 91 | 5 | 5 |
| 93 | 5 | 5 |

15

The conclusion of the results of tables 21-24 is that the peptide sequence can be modified at several positions and by that increase or keep the microbicidal activity compared with the natural sequence. These modified sequences also reduce the costs for synthesis of the peptides. The positions at which amino acids can be changed with better or equal results are the ones denoted Xaa in SEQ. ID. NO. 99.

20

CLAIMS

1. A peptide based on the sequence constituted of the amino acids 12-40 of human lactoferrin counted from the N-terminal end, a modified version thereof, or a
5 fragment thereof, the sequence of which is SEQ. ID. NO. 1 given in the appended sequence listing wherein X in position 1 is E or none, X in position 2 is A or none, X in position 5 is C or A, X in position 7 is Q or K, X in position 11 is N or D, and X in positions 17-25 are -G-P-P-
10 V-S-C-I-K-R or none, or functionally equivalent homologues or analogues thereof.

2. A peptide according to claim 1, wherein X in position 1 is E, X in position 2 is A, X in position 5 is C, X in position 7 is Q, X in position 11 is N, and X in
15 positions 17-25 are -G-P-P-V-S-C-I-K-R, the sequence thus being SEQ. ID. NO. 2 given in appended sequence listing, or functionally equivalent homologues or analogues thereof.

3. A peptide according to claim 2 which is cyclised
20 through a disulphide bridge, the sequence thus being SEQ. ID. NO. 3 given in the appended sequence listing, or functionally equivalent homologues or analogues thereof.

4. A peptide according to claim 1, wherein X in position 1 is none, X in position 2 is none, X in position
25 5 is C, X in position 7 is Q, X in position 11 is N, and X in positions 17-25 are is -G-P-P-V-S-C-I-K-R, the sequence thus being SEQ. ID. NO. 4 given in the appended sequence listing, or functionally equivalent homologues or analogues thereof.

30 5. A peptide according to claim 4 which is cyclised through a disulphide bridge, the sequence thus being SEQ. ID. NO. 5 given in the appended sequence listing, or functionally equivalent homologues or analogues thereof.

6. A peptide based on the sequence constituted of
35 the amino acids 12-40 of human lactoferrin counted from the N-terminal end, a modified version thereof, or a

fragment thereof, the sequence of which is, wherein X in position 1 is none, X in position 2 is none, X in position 5 is A, X in position 7 is K, X in position 11 is D, and X in positions 17-25 are none, the sequence thus being SEQ. ID. NO. 6 given in the appended sequence listing, or functionally equivalent homologues or analogues thereof.

7. A peptide according to claim 6, wherein a lactam bridge is formed between the amino acids K in position 5 and D in position 9, the sequence thus being SEQ. ID. NO. 7 given in the appended sequence listing, or functionally equivalent homologues or analogues thereof.

8. A peptide based on the sequence constituted of the amino acids 12-40 of human lactoferrin counted from the N-terminal end, a modified version thereof, or a fragment thereof, the sequence of which is a fragment of SEQ. ID. NO. 8 given in appended the sequence listing corresponding to SEQ. ID. NO. 31 given in the appended sequence listing, or functionally equivalent homologues or analogues thereof.

9. A peptide based on the sequence constituted of the amino acids 12-40 of human lactoferrin counted from the N-terminal end, a modified version thereof, or a fragment thereof, the sequence of which is a fragment of SEQ. ID. NO. 8 given in appended the sequence listing corresponding to any of SEQ. ID. NO. 31-38 given in the appended sequence listing, or functionally equivalent homologues or analogues thereof.

10. A peptide having one of the sequences SEQ. ID. NO. 46, 48, 50, 52, 54, 56, 58, 60, 62, 64 or 66, or functionally equivalent homologues or analogues thereof.

11. A peptide having one of the sequences SEQ. ID. NO. 50, 52, 54, 56, 60 or 98, or functionally equivalent homologues or analogues thereof.

12. A peptide according to claim 10 or 11, wherein the N-terminal amino acid is acetylated and/or the C-terminal amino acid is amidated.

13. A peptide having one of the sequences SEQ. ID. NO. 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65 or 67, or functionally equivalent homologues or analogues thereof.

14. A peptide having one of the sequences SEQ. ID. NO. 51, 53, 55, 57, 59 61 or 97, or functionally equivalent homologues or analogues thereof.

15. A peptide having a sequence essentially corresponding to one of SEQ. ID. NO. 46-51 and 62-67 but wherein the cysteine has been replaced by an acetamidomethyl-cysteine, or functionally equivalent homologues or analogues thereof.

16. A peptide based on the sequence constituted of the amino acids 12-40 of human lactoferrin counted from the N-terminal end, a modified version thereof, or a fragment thereof, consisting of 12 amino acids, the sequence of which is SEQ. ID. NO. 99, wherein Xaa in position 3 is preferably Gln or Ala, Xaa in position 4 is preferably Trp or Leu, Xaa in position 5 is preferably Gln, Lys, Orn, Ala or Nle, Xaa in position 6 is preferably Arg, Lys or Ala, Xaa in position 7 is preferably Asn, Orn, Ala or Nle, Xaa in position 8 is preferably Met or Leu, and Xaa in position 9 is preferably Arg or Lys.

17. A peptide according to claim 16, the sequence of which is any of SEQ. ID. NO. 68-96 given in the appended sequence listing.

18. A peptide according to claim 17, having SEQ. ID. NO. 70 or SEQ. ID. NO. 74.

19. A peptide according to claim 17, having SEQ. ID. NO. 81 or SEQ. ID. NO. 83.

20. A peptide according to claim 17, having SEQ. ID. NO. 87 or SEQ. ID. NO. 89.

21. A peptide according to claim 17, having SEQ. ID. NO. 91.

22. A medicinal product comprising a peptide according to any of the claims 1-21.

23. A medicinal product according to claim 22 for treatment and/or prevention of infections and/or inflammations.

24. A medicinal product according to claim 23, for
5 treatment and/or prevention of urinary tract infection.

25. A medicinal product according to claim 23, for treatment and/or prevention of colitis.

26. A medicinal product according to claim 23, for treatment of a candida infection on a mucosal membrane.

10 27. A medicinal product according to any one of the claims 22-27 formulated for oral administration.

28. A medicinal product according to any one of the claims 22-27 formulated for parenteral administration.

29. A medicinal product according to claim 28 formu-
15 lated for topical administration.

30. A medicinal product according to any one of the claims 22-29 formulated for administration on mucosal membranes.

31. Food stuff comprising a peptide according to any
20 of the claims 1-21.

32. Food stuff according to claim 31 being an infant formula food.

33. Use of a peptide according to any one of the claims 1-21 for the production of a medicinal product for
25 treatment and/or prevention of infections and/or inflammations.

34. Use according to claim 33, wherein the medicinal product is intended for treatment and/or prevention of urinary tract infection.

30 35. Use according to claim 33, wherein the medicinal product is intended for treatment and/or prevention of colitis.

36. Use according to claim 33, wherein the medicinal product is intended for treatment of a candida infection
35 on a mucosal membrane.

37. Use according to any one of the claims 33-36, wherein the medicinal product is formulated for oral administration.

38. Use according to any one of the claims 33-36, wherein the medicinal product is formulated for parenteral administration.

39. Use according to claim 38, wherein the medicinal product is formulated for topical administration.

40. Use according to any one of the claims 33-39, wherein the medicinal product is formulated for administration on mucosal membranes.

41. Use according to claim 37, wherein the medicinal product constitutes or is included in a food stuff.

42. Use according to claim 41, wherein the food stuff is an infant formula food.

43. A method for treatment or prevention of infections or inflammations wherein an effective amount of a substance chosen from the group consisting of the peptides having any of the sequences SEQ ID NO 1 - 7, 31 - 38, or 46 - 99, fragments, and functionally equivalent homologues and analogues thereof, is administered to a patient.

44. A method according to claim 43 for treatment and/or prevention of urinary tract infection.

45. A method according to claim 43 for treatment and/or prevention of colitis.

46. A method according to claim 43 for treatment and/or prevention of a candida infection on a mucosal membrane.

47. A method according to claim 43, wherein the substance is orally administered.

48. A method according to claim 43, wherein the substance is parenterally administered.

49. A method according to claim 48, wherein the substance is topically administered.

50. A method according to claim 49, wherein the substance is administered on mucosal membranes.

47

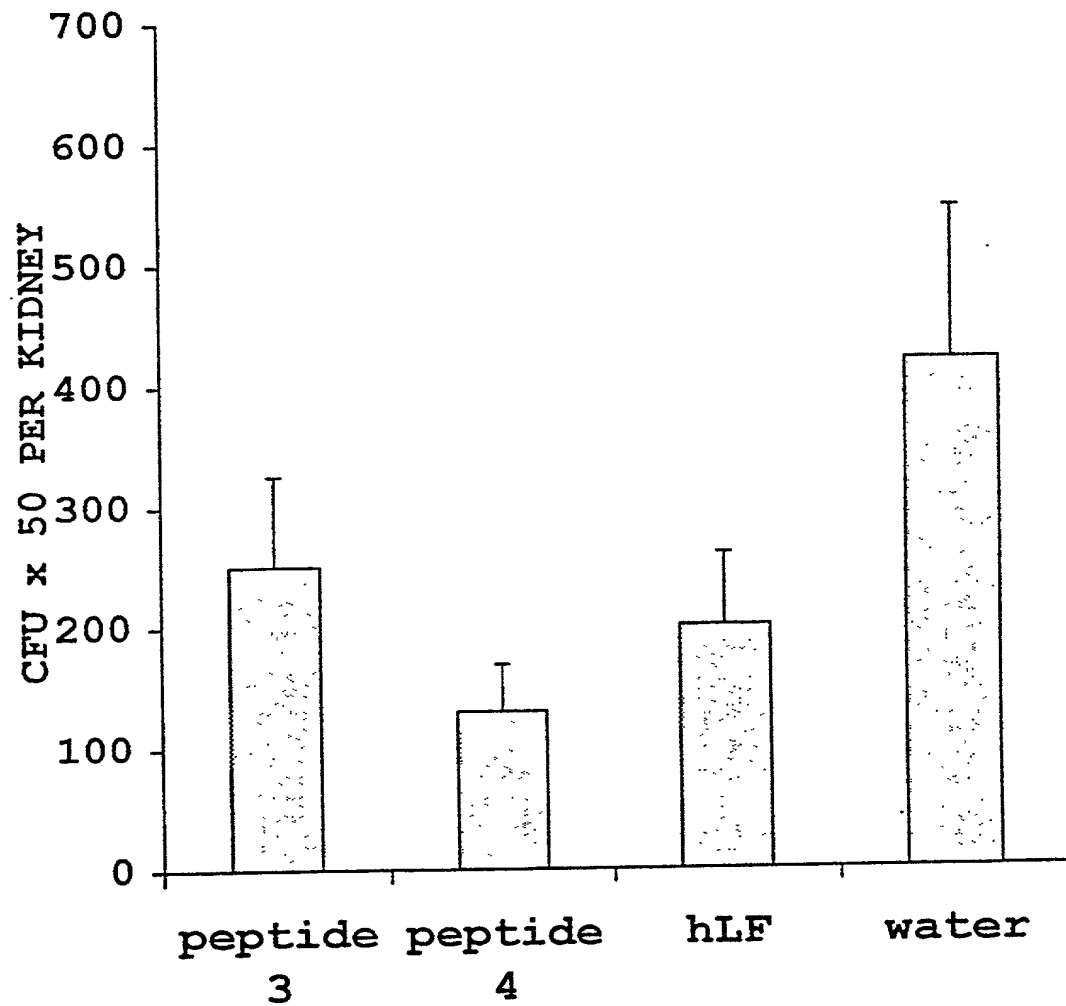
51. A method according to claim 47, wherein the substance is included in a food stuff.

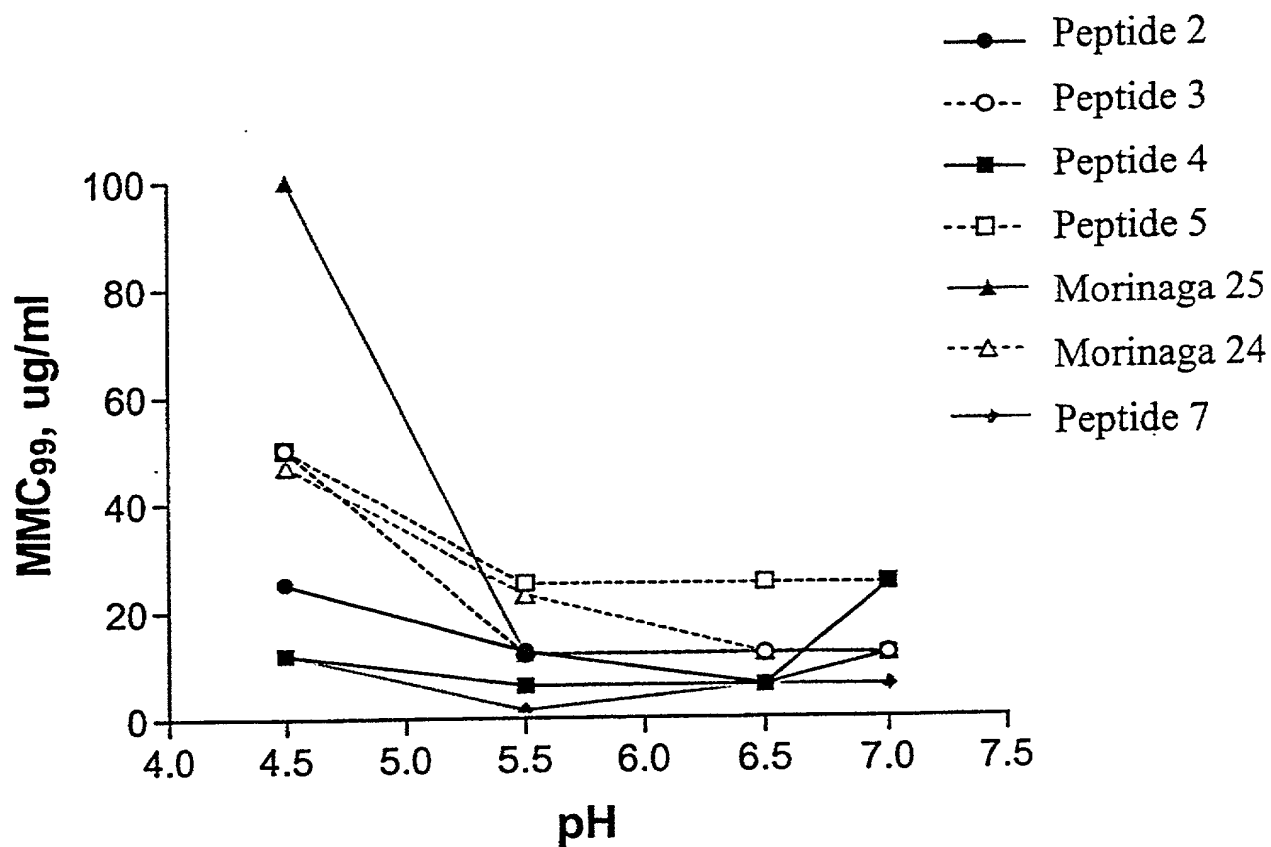
52. A method according to claim 51, wherein the substance is included in an infant formula food.

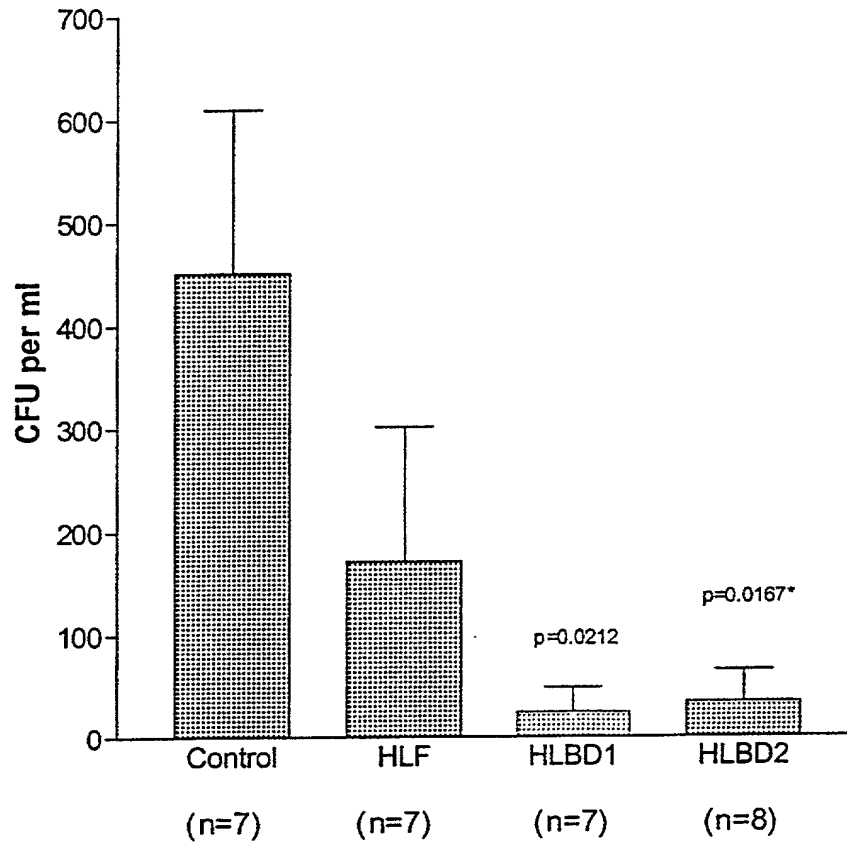
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AMENDED SHEET

AMENDED SHEET
The present sheet is a replacement for the original sheet
No. 1 of the International Publication No. 99/01230

*Figure 1*

*Fig. 2*

C. albicans in stomach*Fig. 3*

Colitis in C57Bl/6 mice

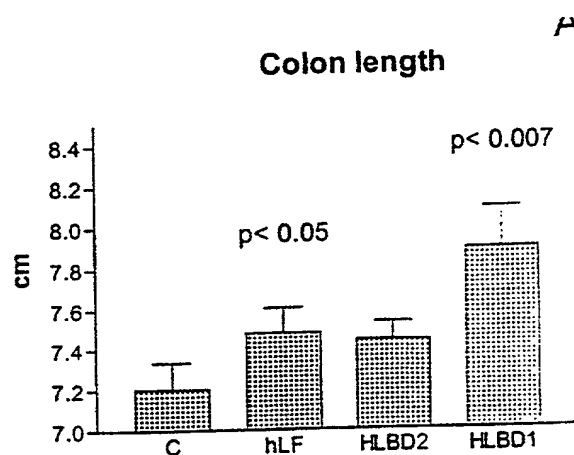
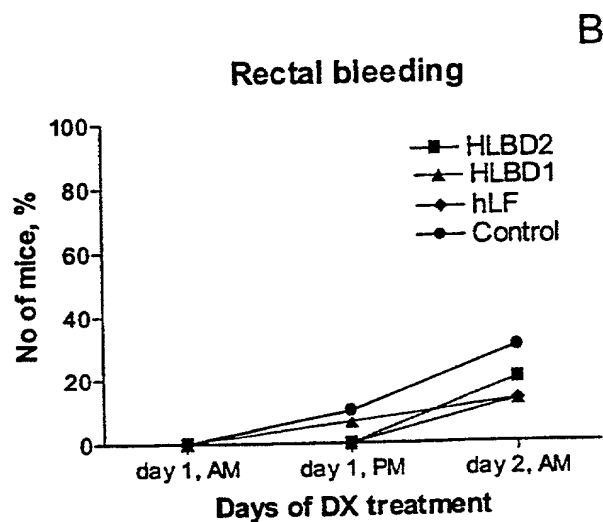
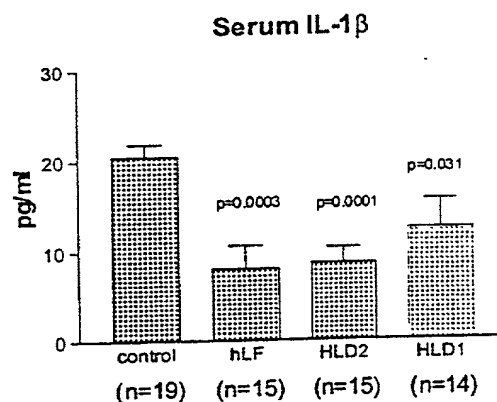
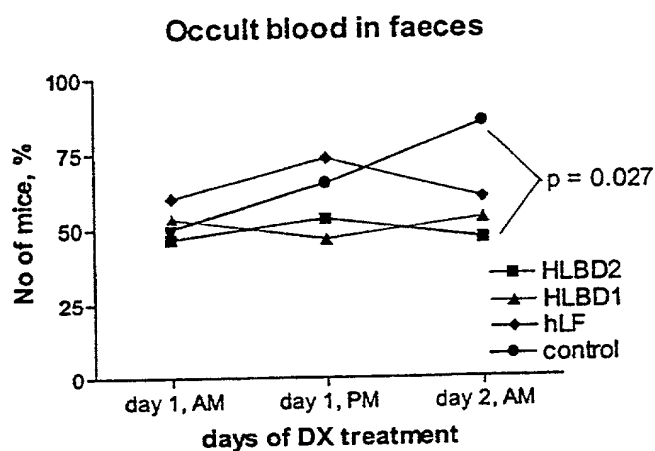
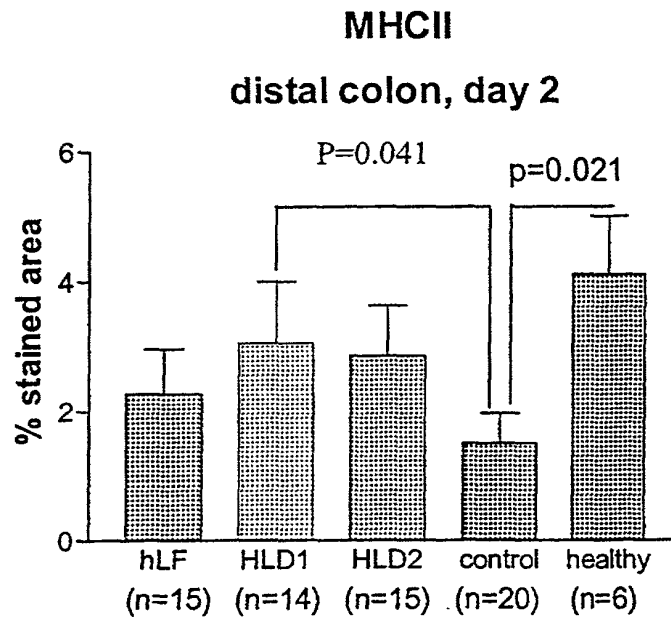
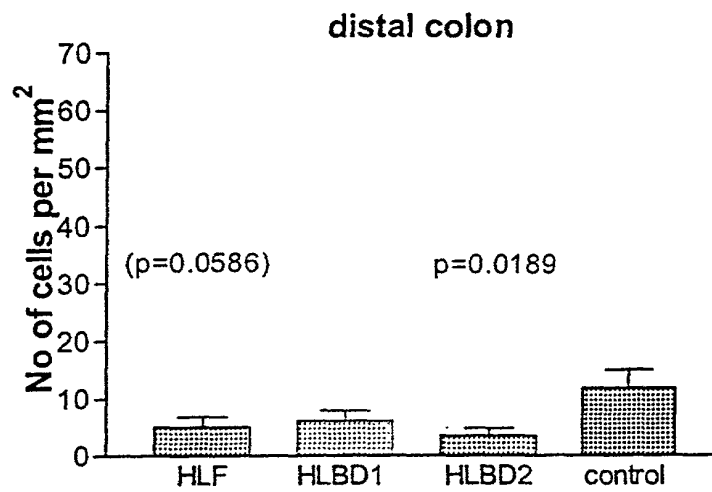


Fig. 4

5/5

Colitis CD8, day 2

**Fig. 5**

**COMBINED DECLARATION AND POWER OF ATTORNEY
FOR UTILITY PATENT APPLICATION**

Attorney's Docket No.

003300-723

As a below-named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name;

I BELIEVE I AM THE ORIGINAL, FIRST AND SOLE INVENTOR (if only one name is listed below) OR AN ORIGINAL, FIRST AND JOINT INVENTOR (if more than one name is listed below) OF THE SUBJECT MATTER WHICH IS CLAIMED AND FOR WHICH A PATENT IS SOUGHT ON THE INVENTION ENTITLED:

PEPTIDES BASED ON THE SEQUENCE OF HUMAN LACTOFERRIN AND THEIR USE

the specification of which

(check one)

☐ is attached hereto;

☒ was filed on July 6, 1999 as

Application No. PCT/SE99/01230

and was amended on September 29, 2000;
(if applicable)

I HAVE REVIEWED AND UNDERSTAND THE CONTENTS OF THE ABOVE-IDENTIFIED SPECIFICATION, INCLUDING THE CLAIMS, AS AMENDED BY ANY AMENDMENT REFERRED TO ABOVE;

I ACKNOWLEDGE THE DUTY TO DISCLOSE TO THE OFFICE ALL INFORMATION KNOWN TO ME TO BE MATERIAL TO PATENTABILITY AS DEFINED IN TITLE 37, CODE OF FEDERAL REGULATIONS, Sec. 1.56 (as amended effective March 16, 1992);

I do not know and do not believe the said invention was ever known or used in the United States of America before my or our invention thereof, or patented or described in any printed publication in any country before my or our invention thereof or more than one year prior to said application; that said invention was not in public use or on sale in the United States of America more than one year prior to said application; that said invention has not been patented or made the subject of an inventor's certificate issued before the date of said application in any country foreign to the United States of America on any application filed by me or my legal representatives or assigns more than twelve months prior to said application;

I hereby claim foreign priority benefits under Title 35, United States Code Sec. 119 and/or Sec. 365 of any foreign application(s) for patent or inventor's certificate as indicated below and have also identified below any foreign application for patent or inventor's certificate on this invention having a filing date before that of the application(s) on which priority is claimed:

COMBINED DECLARATION AND POWER OF ATTORNEY

Attorney's Docket No.

003300-723

| COUNTRY/INTERNATIONAL | APPLICATION NUMBER | DATE OF FILING (day, month, year) | PRIORITY CLAIMED |
|-----------------------|--------------------|--------------------------------------|---------------------------|
| Sweden | 9802441-7 | 6 July 1998 | YES <u>X</u> NO <u> </u> |
| Sweden | 9802562-0 | 17 July 1998 | YES <u>X</u> NO <u> </u> |
| Sweden | 9804614-7 | 29 December 1998 | YES <u>X</u> NO <u> </u> |

I hereby appoint the following attorneys and agent(s) to prosecute said application and to transact all business in the Patent and Trademark Office connected therewith and to file, prosecute and to transact all business in connection with international applications directed to said invention:

William L. Mathis 17,337
Robert S. Swecker 19,885
Platon N. Mandros 22,124
Benton S. Duffett, Jr. 22,030
Norman H. Stepano 22,716
Ronald L. Grudziecki 24,970
Frederick G. Michaud, Jr. 26,003
Alan E. Kopecki 25,813
Regis E. Slutter 26,999
Samuel C. Miller, III 27,360
Robert G. Mukai 28,531
George A. Hovanec, Jr. 28,223
James A. LaBarre 28,632
E. Joseph Gess 28,510
R. Danny Huntington 27,903

Eric H. Weisblatt 30,505
James W. Peterson 26,057
Teresa Stanek Rea 30,427
Robert E. Krebs 25,885
William C. Rowland 30,888
T. Gene Dillahunty 25,423
Patrick C. Keane 32,858
B. Jefferson Boggs, Jr. 32,344
William H. Benz 25,952
Peter K. Skiff 31,917
Richard J. McGrath 29,195
Matthew L. Schneider 32,814
Michael G. Savage 32,596
Gerald F. Swiss 30,113
Charles F. Wicland III 33,096

Bruce T. Wieder 33,815
Todd R. Walters 34,040
Rommi S. Jillions 31,979
Harold R. Brown III 36,341
Allen R. Baum 36,086
Steven M. duBois 35,023
Brian P. O'Shaughnessy 32,747
Kenneth B. Leffler 36,075
Fred W. Hathaway 32,236
Wendi L. Weinstein 34,456
Mary Ann Dillahunty 34,576



21839

and: none

Address all correspondence to:



21839

Benton S. Duffett, Jr.

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

P.O. Box 1404

Alexandria, Virginia 22313-1404

Address all telephone calls to: Benton S. Duffett, Jr. at (703) 836-6620.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

FULL NAME OF SOLE OR FIRST INVENTOR

LARS A. HANSON

SIGNATURE

DATE

01/02/01

RESIDENCE

Göteborg, Sweden

CITIZENSHIP

Sweden

POST OFFICE ADDRESS

Västergatan 2, SE-411 26 Göteborg, Sweden

FULL NAME OF SECOND JOINT INVENTOR, IF ANY

LARS BALTZER

SIGNATURE

DATE

01/02/01

RESIDENCE

Göteborg, Sweden

CITIZENSHIP

Sweden

POST OFFICE ADDRESS

Förtroligheten 23, SE-412 70 Göteborg, Sweden

COMBINED DECLARATION AND POWER OF ATTORNEY

Attorney's Docket No.

003300-723

3-00 FULL NAME OF THIRD JOINT INVENTOR, IF ANY

SIGNATURE

DATE

INGER MATTSBY-BALTZER

RESIDENCE

Göteborg, Sweden

SEX

CITIZENSHIP

Sweden

POST OFFICE ADDRESS

Förtroligheten 23, SE-412 70 Göteborg, Sweden

4-00 FULL NAME OF FOURTH JOINT INVENTOR, IF ANY

SIGNATURE

DATE

GUNNAR T. DOLPHIN

RESIDENCE

Göteborg, Sweden

SEX

CITIZENSHIP

Sweden

POST OFFICE ADDRESS

Skytteskogsgatan 14, SE-414 76 Göteborg, Sweden

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SEQUENCE LISTING

<110> A+ Science Invest AB
Mattsby-Baltzer, Inger
Hanson Å, Lars
Dolphin T, Gunnar

<120> NEW PEPTIDES AND USE THEREOF

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Pro Val Ser Cys Ile Lys Arg
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Pro Val Ser Cys Ile Lys Arg
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lactoferrin

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Thr Lys Ala Phe Lys Trp Gln Arg Asp Met Arg Lys Val Arg
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<210> 7

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lactoferrin; a lactam is formed between aa 5 and 9

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Val Ser Gln Pro Glu Ala Thr Lys Cys Phe Gln Trp Gln Arg Asn Met
1 5 10 15

Arg Lys Val Arg
20

<210> 9

<211> 7

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consisting of aa 12-18 in human lactoferrin

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Val Ser Gln Pro Glu Ala Thr
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<210> 10

<211> 7

<212> PRT

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consisting of aa 13-19 in human lactoferrin

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Ser Gln Pro Glu Ala Thr Lys

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<210> 12

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<400> 12

Pro Glu Ala Thr Lys Cys Phe

1

5

<210> 13

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Glu Ala Thr Lys Cys Phe Gln

1

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consisting of aa 17-23 in human lactoferrin

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Ala Thr Lys Cys Phe Gln Trp

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<223> Description of Artificial Sequence: of natural or
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consisting of aa 18-24 in human lactoferrin

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Thr Lys Cys Phe Gln Trp Gln

1

5

<210> 16

<211> 7

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<223> Description of Artificial Sequence: of natural or

artificial origin, corresp. to the sequence
consisting of aa 19-25 in human lactoferrin

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Lys Cys Phe Gln Trp Gln Arg

1

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<211> 7

<212> PRT

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consisting of aa 20-26 in human lactoferrin

<400> 17

Cys Phe Gln Trp Gln Arg Asn

1

5

<210> 18

<211> 7

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<400> 18

Phe Gln Trp Gln Arg Asn Met

1

5

<210> 19

<211> 7

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artificial origin, corresp. to the sequence
consisting of aa 22-28 in human lactoferrin

<400> 19

Gln Trp Gln Arg Asn Met Arg

1

5

<210> 20

<211> 7

<212> PRT

<213> Artificial Sequence

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<223> Description of Artificial Sequence: of natural or
artificial origin, corresp. to the sequence
consisting of aa 23-29 in human lactoferrin

<400> 20

Trp Gln Arg Asn Met Arg Lys

1

5

<210> 21

<211> 7

<212> PRT

<213> Artificial Sequence

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<400> 21

Gln Arg Asn Met Arg Lys Val

1

5

<210> 22

<211> 7

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<400> 22

Arg Asn Met Arg Lys Val Arg

1

5

<210> 23

<211> 8

<212> PRT

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Glu Ala Thr Lys Cys Phe Gln Trp

1

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<211> 9

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Glu Ala Thr Lys Cys Phe Gln Trp Gln

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5

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Glu Ala Thr Lys Cys Phe Gln Trp Gln Arg

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consisting of aa 16-26 in human lactoferrin

<400> 26

Glu Ala Thr Lys Cys Phe Gln Trp Gln Arg Asn

1 5 10

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Glu Ala Thr Lys Cys Phe Gln Trp Gln Arg Asn Met

1 5 10

<210> 28

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<400> 28

Glu Ala Thr Lys Cys Phe Gln Trp Gln Arg Asn Met Arg
1 5 10

<210> 29

<211> 14

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<400> 29

Glu Ala Thr Lys Cys Phe Gln Trp Gln Arg Asn Met Arg Lys
1 5 10

<210> 30

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<400> 30

Glu Ala Thr Lys Cys Phe Gln Trp Gln Arg Asn Met Arg Lys Val
1 5 10 15

<210> 31

<211> 16

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consisting of aa 16-31 in human lactoferrin

<400> 31

Glu Ala Thr Lys Cys Phe Gln Trp Gln Arg Asn Met Arg Lys Val Arg
1 5 10 15

<210> 32

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Ser Gln Pro Glu Ala Thr Lys Cys Phe Gln Trp Gln Arg Asn Met Arg
1 5 10 15

Lys Val Arg

<210> 33

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Gln Pro Glu Ala Thr Lys Cys Phe Gln Trp Gln Arg Asn Met Arg Lys
1 5 10 15

Val Arg

<210> 34

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Pro Glu Ala Thr Lys Cys Phe Gln Trp Gln Arg Asn Met Arg Lys Val
1 5 10 15

Arg

<210> 35

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Ala Thr Lys Cys Phe Gln Trp Gln Arg Asn Met Arg Lys Val Arg
1 5 10 15

<210> 36

<211> 14

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Thr Lys Cys Phe Gln Trp Gln Arg Asn Met Arg Lys Val Arg
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Lys Cys Phe Gln Trp Gln Arg Asn Met Arg Lys Val Arg
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Cys Phe Gln Trp Gln Arg Asn Met Arg Lys Val Arg
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Trp Gln Arg Asn Met Arg Lys Val Arg
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<210> 42
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Phe Xaa Trp Xaa Arg Xaa Met Arg Lys Xaa Arg
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1 5 10

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Cys Phe Gln Trp Gln Arg Asn Met Arg Lys Val Arg

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<210> 47

<211> 12

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Lys Cys Phe Gln Trp Gln Arg Asn Met Arg Lys Val Arg
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<210> 50

<211> 14

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1 5 10

<210> 51

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lactoferrin

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lactoferrin; a lactam is formed between aa 5 and 9

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5

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<210> 55

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<210> 56

<211> 14

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: of natural or artificial origin, corresp. to a modification of the sequence consisting of aa 18-31 in human lactoferrin

<400> 56

Thr Lys Lys Phe Gln Trp Gln Arg Asn Met Arg Lys Val Arg
 1 5 10

<210> 57

<211> 14

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: of natural or
 artificial origin, corresp. to a modification of
 the sequence consisting of aa 18-31 in human
 lactoferrin

<220>

<221> MOD_RES

<222> (1)

<223> ACETYLTATION

<220>

<221> MOD_RES

<222> (14)

<223> AMIDATION

<400> 57

Thr Lys Lys Phe Gln Trp Gln Arg Asn Met Arg Lys Val Arg
 1 5 10

<210> 58

<211> 14

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: of natural or
 artificial origin, corresp. to a modification of
 the sequence consisting of aa 18-31 in human
 lactoferrin

<400> 58

Thr Lys Lys Phe Gln Trp Asp Arg Lys Met Arg Lys Asp Arg
 1 5 10

<210> 59
 <211> 14
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: of natural or
 artificial origin, corresp. to a modification of
 the sequence consisting of aa 18-31 in human
 lactoferrin

<220>
 <221> MOD_RES
 <222> (1)
 <223> ACETYLATION

<220>
 <221> MOD_RES
 <222> (14)
 <223> AMIDATION

<400> 59
 Thr Lys Lys Phe Gln Trp Asp Arg Lys Met Arg Lys Asp Arg
 1 5 10

<210> 60
 <211> 14
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: of natural or
 artificial origin, corresp. to a modification of
 the seq. consisting of aa 18-31 in human
 lactoferrin; lactams formed between aa 3 and 7,
 and 9 and 13

<220>
 <221> BINDING
 <222> (3) .. (7)
 <223> LACTAM

<220>
 <221> BINDING
 <222> (9) .. (13)

<223> LACTAM

<400> 60

Thr Lys Lys Phe Gln Trp Asp Arg Lys Met Arg Lys Asp Arg
1 5 10

<210> 61

<211> 14

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: of natural or
artificial origin, corresp. to a modification of
the seq. consisting of aa 18-31 in human
lactoferrin; lactams formed between aa 3 and 7,
and 9 and 13

<220>

<221> MOD_RES

<222> (1)

<223> ACETYLATION

<220>

<221> MOD_RES

<222> (14)

<223> AMIDATION

<220>

<221> BINDING

<222> (3) .. (7)

<223> LACTAM

<220>

<221> BINDING

<222> (9) .. (13)

<400> 61

Thr Lys Lys Phe Gln Trp Asp Arg Lys Met Arg Lys Asp Arg
1 5 10

<210> 62

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: of natural or artificial origin, corresp. to the sequence consisting of aa 17-31 in human lactoferrin

<400> 62

| | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Thr | Lys | Cys | Phe | Gln | Trp | Gln | Arg | Asn | Met | Arg | Lys | Val | Arg |
| 1 | | | | 5 | | | | 10 | | | | | 15 | |

<210> 63

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: of natural or artificial origin, corresp. to a modification of the sequence consisting of aa 17-31 in human lactoferrin

<220>

<221> MOD_RES

<222> (1)

<223> ACETYLTATION

<220>

<221> MOD_RES

<222> (15)

<223> AMIDATION

<400> 63

| | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Thr | Lys | Cys | Phe | Gln | Trp | Gln | Arg | Asn | Met | Arg | Lys | Val | Arg |
| 1 | | | | 5 | | | | 10 | | | | | 15 | |

<210> 64

<211> 16

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: of natural or artificial origin, corresp. to the sequence

consisting of aa 16-31 in human lactoferrin

<400> 64

Glu Ala Thr Lys Cys Phe Gln Trp Gln Arg Asn Met Arg Lys Val Arg
1 5 10 15

<210> 65

<211> 16

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: of natural or
artificial origin, corresp. to a modification of
the sequence consisting of aa 16-31 in human
lactoferrin

<220>

<221> MOD_RES

<222> (1)

<223> ACETYLTATION

<220>

<221> MOD_RES

<222> (16)

<223> AMIDATION

<400> 65

Glu Ala Thr Lys Cys Phe Gln Trp Gln Arg Asn Met Arg Lys Val Arg
1 5 10 15

<210> 66

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: of natural or
artificial origin, corresp. to the sequence
consisting of aa 15-31 in human lactoferrin

<400> 66

Pro Glu Ala Thr Lys Cys Phe Gln Trp Gln Arg Asn Met Arg Lys Val
1 5 10 15

Arg

<210> 67

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: of natural or
artificial origin, corresp. to a modification of
the sequence consisting of aa 15-31 in human
lactoferrin

<220>

<221> MOD_RES

<222> (1)

<223> ACETYLTATION

<220>

<221> MOD_RES

<222> (17)

<223> AMIDATION

<400> 67

Pro Glu Ala Thr Lys Cys Phe Gln Trp Gln Arg Asn Met Arg Lys Val
1 5 10 15

Arg

<210> 68

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: of natural or
artificial origin, corresp. to the sequence
consisting of aa 20-31 in human lactoferrin
wherein one aa has been substituted

<400> 68

Ala Phe Gln Trp Gln Arg Asn Met Arg Lys Val Arg
1 5 10

<210> 69

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: of natural or
artificial origin, corresp. to the sequence
consisting of aa 20-31 in human lactoferrin
wherein one aa has been substituted

<400> 69

Cys Ala Gln Trp Gln Arg Asn Met Arg Lys Val Arg
1 5 10

<210> 70

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: of natural or
artificial origin, corresp. to the sequence
consisting of aa 20-31 in human lactoferrin
wherein one aa has been substituted

<400> 70

Cys Phe Ala Trp Gln Arg Asn Met Arg Lys Val Arg
1 5 10

<210> 71

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: of natural or
artificial origin, corresp. to the sequence
consisting of aa 20-31 in human lactoferrin

wherein one aa has been substituted

<400> 71

Cys Phe Gln Ala Gln Arg Asn Met Arg Lys Val Arg

1 5 10

<210> 72

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: of natural or
artificial origin, corresp. to the sequence
consisting of aa 20-31 in human lactoferrin,
wherein one aa has been substituted

<400> 72

Cys Phe Gln Trp Ala Arg Asn Met Arg Lys Val Arg

1 5 10

<210> 73

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: of natural or
artificial origin, corresp. to the sequence
consisting of aa 20-31 in human lactoferrin
wherein one aa has been substituted

<400> 73

Cys Phe Gln Trp Gln Ala Asn Met Arg Lys Val Arg

1 5 10

<210> 74

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: of natural or artificial origin, corresp. to the sequence consisting of aa 20-31 in human lactoferrin wherein one aa has been substituted

<400> 74

Cys Phe Gln Trp Gln Arg Ala Met Arg Lys Val Arg
1 5 10

<210> 75

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: of natural or artificial origin, corresp. to the sequence consisting of aa 20-31 in human lactoferrin wherein one aa had been substituted

<400> 75

Cys Phe Gln Trp Gln Arg Asn Ala Arg Lys Val Arg
1 5 10

<210> 76

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: of natural or artificial origin, corresp. to the sequence consisting of aa 20-31 in human lactoferrin wherein one aa has been substituted

<400> 76

Cys Phe Gln Trp Gln Arg Asn Met Ala Lys Val Arg
1 5 10

<210> 77

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: of natural or artificial origin, corresp. to the sequence consisting of aa 20-31 in human lactoferrin wherein one aa has been substituted

<400> 77

Cys Phe Gln Trp Gln Arg Asn Met Arg Ala Val Arg
1 5 10

<210> 78

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: of natural or artificial origin, corresp. to the sequence consisting of aa 20-31 in human lactoferrin wherein one aa has been substituted

<400> 78

Cys Phe Gln Trp Gln Arg Asn Met Arg Lys Ala Arg
1 5 10

<210> 79

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: of natural or artificial origin, corresp. to the sequence consisting of aa 20-31 in human lactoferrin wherein one aa has been substituted

<400> 79

Cys Phe Gln Trp Gln Arg Asn Met Arg Lys Val Ala
1 5 10

<210> 80
<211> 12
<212> PRT
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: of natural or
artificial origin, corresp. to the sequence
consisting of aa 20-31 in human lactoferrin
wherein one aa has been substituted

<400> 80

Cys Phe Gln Leu Gln Arg Asn Met Arg Lys Val Arg
1 5 10

<210> 81
<211> 12
<212> PRT
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: of natural or
artificial origin, corresp. to the sequence
consisting of aa 20-31 in human lactoferrin
wherein one aa has been substituted

<400> 81

Cys Phe Gln Trp Gln Lys Asn Met Arg Lys Val Arg
1 5 10

<210> 82
<211> 12
<212> PRT
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: of natural or
artificial origin, corresp. to the sequence
consisting of aa 20-31 in human lactoferrin
wherein one aa has been substituted

<400> 82

Cys Phe Gln Trp Gln Arg Asn Leu Arg Lys Val Arg
1 5 10

<210> 83
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: of natural or
artificial origin, corresp. to the sequence
consisting of aa 20-31 in human lactoferrin
wherein one aa has been substituted

<400> 83
Cys Phe Gln Trp Gln Arg Asn Met Lys Lys Val Arg
1 5 10

<210> 84
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: of natural or
artificial origin, corresp. to the sequence
consisting of aa 20-31 in human lactoferrin
wherein one aa has been substituted

<400> 84
Cys Phe Gln Trp Glu Arg Asn Met Arg Lys Val Arg
1 5 10

<210> 85
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: of natural or
artificial origin, corresp. to the sequence
consisting of aa 20-31 in human lactoferrin
wherein one aa has been substituted

<400> 85

Cys Phe Gln Trp Gln Glu Asn Met Arg Lys Val Arg
1 5 10

<210> 86

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: of natural or
artificial origin, corresp. to the sequence
consisting of aa 20-31 in human lactoferrin
wherein one aa has been substituted

<400> 86

Cys Phe Gln Trp Gln Arg Glu Met Arg Lys Val Arg
1 5 10

<210> 87

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: of natural or
artificial origin, corresp. to the sequence
consisting of aa 20-31 in human lactoferrin
wherein one aa has been substituted

<220>

<223> Xaa in position 5 is Orn

<400> 87

Cys Phe Gln Trp Xaa Arg Asn Met Arg Lys Val Arg
1 5 10

<210> 88

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: of natural or artificial origin, corresp. to the sequence consisting of aa 20-31 in human lactoferrin wherein one aa has been substituted

<220>

<223> Xaa in position 5 is Nle

<400> 88

| | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Cys | Phe | Gln | Trp | Xaa | Arg | Asn | Met | Arg | Lys | Val | Arg |
| 1 | | | | 5 | | | | | 10 | | |

<210> 89

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: of natural or artificial origin, corresp. to the sequence consisting of aa 20-31 in human lactoferrin wherein one aa has been substituted

<220>

<223> Xaa in position 7 is Orn

<400> 89

| | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Cys | Phe | Gln | Trp | Gln | Arg | Xaa | Met | Arg | Lys | Val | Arg |
| 1 | | | | 5 | | | | | 10 | | |

<210> 90

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: of natural or artificial origin, corresp. to the sequence consisting of aa 20-31 in human lactoferrin wherein one aa has been substituted

<220>

<223> Xaa in position 7 is Nle

<400> 90

Cys Phe Gln Trp Gln Arg Xaa Met Arg Lys Val Arg
1 5 10

<210> 91

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: of natural or
artificial origin, corresp. to the sequence
consisting of aa 20-31 in human lactoferrin
wherein one aa has been substituted

<400> 91

Cys Phe Gln Trp Lys Arg Asn Met Arg Lys Val Arg
1 5 10

<210> 92

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: of natural or
artificial origin, corresp. to a modification of
the sequence consisting of aa 18-31 in human
lactoferrin wherein one aa has been substituted

<220>

<221> MOD_RES

<222> (1)

<223> ACETYLTATION

<220>

<221> MOD_RES

<222> (12)

<223> AMIDATION

<400> 92

Cys Phe Gln Trp Lys Arg Asn Met Arg Lys Val Arg
1 5 10

<223> Description of Artificial Sequence: of natural or artificial origin, corresp. to the sequence consisting of aa 20-31 in human lactoferrin wherein some aa have been substituted

Cys Phe Gln Trp Lys Arg Ala Met Arg Lys Val Arg
1 5 10

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<210> 94
<211> 12
<212> PRT
<213> Artificial Sequence
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<223> Description of Artificial Sequence: of natural or artificial origin, corresp. to the sequence consisting of aa 20-31 in human lactoferrin wherein some aa have been substituted

Cys Phe Ala Trp Lys Arg Asn Met Arg Lys Val Arg
1 5 10

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<210> 95
<211> 12
<212> PRT
<213> Artificial Sequence
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<223> Description of Artificial Sequence: of natural or artificial origin, corresp. to the sequence consisting of aa 20-31 in human lactoferrin wherein some aa have been substituted

<400> 95

Cys Phe Ala Trp Gln Arg Ala Met Arg Lys Val Arg

1

5

10

<210> 96

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: of natural or
artificial origin, corresp. to the sequence
consisting of aa 20-31 in human lactoferrin
wherein some aa have been substituted

<400> 96

Cys Phe Gln Leu Gln Lys Asn Met Lys Lys Val Arg

1

5

10

<210> 97

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: of natural or
artificial origin, corresp. to a modification of
the sequence consisting of aa 20-31 in human
lactoferrin

<400> 97

Cys Phe Ala Leu Lys Lys Ala Met Lys Lys Val Arg

1

5

10

<210> 98

<211> 14

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: of natural or
artificial origin, corresp. to a modification of

the sequence consisting of aa 18-31 in human
lactoferrin

<220>

<221> MOD_RES

<222> (1)

<223> ACETYLTATION

<220>

<221> MOD_RES

<222> (14)

<223> AMIDATION

<400> 98

Thr Lys Lys Phe Gln Trp Gln Arg Asn Met Arg Lys Val Arg

1

5

10

<210> 99

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: of natural or
artificial origin, corresp. to a modification of
the sequence consisting of aa 20-31 in human
lactoferrin

<400> 99

Cys Phe Xaa Xaa Xaa Xaa Xaa Xaa Xaa Lys Val Arg

1

5

10

<210> 100

<211> 29

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: a fragment of
human lactoferrin consisting of aa 12-40

<400> 100

Val Ser Gln Pro Glu Ala Thr Lys Cys Phe Gln Trp Gln Arg Asn Met

1

5

10

15

001430-00210

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PCT/SE99/01230

Arg Lys Val Arg Gly Pro Pro Val Ser Cys Ile Lys Arg
20 25

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